EFFECTS OF GROWTH RATES OF HEIFERS AND FEEDING SUPPLEMENTAL GLUCOGENIC PRECURSORS DURING TRANSITION PERIOD ON SOME PHYSIOLOGICAL CHANGES AND MILK PRODUCTION OF HOLSTEIN COWS

By

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By

Tomas I. Belloso

To God, who gave me the strength for the fulfilment of my graduate studies. This dissertation is dedicated to my wife Gabriela, and to my parents Tomas and Zoila, my brothers Mario (Ipe) and Juan Manuel, and my sisters Zoila (Zoy) and Maria Isabel for their unending support and encouragement.

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Abstract of Dissertation Presented to the Graduate School
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EFFECTS OF GROWTH RATES OF HEIFERS AND FEEDING SUPPLEMENTAL GLUCOGENIC PRECURSORS DURING TRANSITION PERIOD ON SOME PHYSIOLOGICAL CHANGES AND MILK PRODUCTION OF HOLSTEIN COWS

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In experiment 1, Holstein heifers that completed 150 days in milk (DIM) (n=88) were used to evaluate effects of diet, season of calving (SEA), and bovine somatotropin (bST) on milk yield (MY), body weight (BW) and body condition score (BCS). They were from a group of 121 heifers raised on four diets that contained different amounts of protein and energy with different energy:protein ratios. One-half the heifers in each diet group were injected biweekly with bST to provide 28 µg of bST/kg/d of BW. At BW>341 Kg all heifers were fed the same diet and bST injections were discontinued. Average daily BW gains (ADGs) to 341 kg BW were 0.854, 0.949, 0.986 and 0.979 kg/d ($P<0.01$). Calving traits, BW and BCS, and mean MY through 150 DIM did not differ and no effects of bST were detected. Heifers calving in cooler SEA produced more milk, but no interaction with treatment was detected. In experiment 2, multiparous Holstein cows were supplemented with glucogenic precursors during 3 wk prepartum through 4 wk postpartum (transition period). Treatments (TRT) were 1) control, none, n=29; 2) NutroCAL™, 0.114 kg/d, n=33; 3) Metaxerol™, 0.454 kg/d, n=31; and 4) propylene glycol, 300 mL/d, n=31. Cows were fed twice daily and DMI recorded during these 7 wk. Body weight and BCS were recorded weekly beginning 21 d prepartum and blood samples were collected thrice weekly. Liver samples were obtained from 40 cows (10/TRT) at -28, calving, +14 and +28 d. Dry matter intake, BW and BCS showed expected changes during the transition period, but DMI declined 24.3% around calving. Postpartum DMI increased during lactation; similar trends were seen for BW and BCS. The MY did not differ among treatments during 4-28, 4-100, 28-100, or 4-150 DIM. Small, but physiologically unimportant differences in BW and BCS, insulin, IGF-1, NEFA, β-HBA, and liver lipids were detected prepartum and/or postpartum across treatments or due to SEA. Overall, cows in all groups had similar MY, maintained BW and BCS equally well, and had similar patterns of DMI during prepartum and postpartum periods.

CHAPTER 1
INTRODUCTION

As costs of milk production continue to increase, dairy farmers face new challenges so their operations can be profitable. Their overall goal is to increase milk production while reducing the costs. This goal can be achieved by improving management and animal selection, by implementing use of improved knowledge of nutrition and physiology, and by reducing metabolic and pathologic diseases that adversely affect cow health and productivity. Clearly, a healthy milking herd is indispensable for success of the dairy farm operation.

The heifer replacement program (HRP) is an important enterprise on many dairy farms. This program provides the heifers that will replace older cows that leave the herd. Success or failure of the HRP also is reflected in the profits that the dairy operation may generate. The costs of raising heifers can account for 15 to 20% of the total milk production costs (Annexstad, 1986) and often are the second largest input, after feed costs, for the milking herd (Goodger et al., 1989).

The organization of activities of the HRP can be divided into four time periods related to the growth cycle of the heifers that are being raised. These are categorized by age into the following time periods: 1) birth to weaning; 2) weaning to puberty; 3) puberty to calving (pregnancy); 4) postcalving or lactation. An important period embedded within the late 3rd phase and early 4th phase described is the transition period. The transition period can be described as that which includes the time from 3 wk before calving through 3 wk after calving. How successfully cows pass through the transition period has great impact on health of cows and success of the lactation that follows calving. In this context, the transition period is important for the passage of cows that previously had lactated and also the first calf heifers.

In the past, more attention was placed on postpartum cows and much less on how well heifers reached the desired age and weight at their first calving (500-550 kg by 22-24 mo of age) and their management during the transition period. Clearly, it is important that both heifers and cows are adequately managed so that they are well-prepared for the physiological stresses imposed by fetal growth, the stress and any associated problems of calving, and the start and maintenance of the lactation.

During the past 20 yr or more, many studies were completed that demonstrated that the recommended levels of nutrients and energy fed to heifers during the growth phase (e.g., NRC, 1989) were not adequate to support normal growth rates desired for the modern Holstein heifer. However, it also was clear that the experiments completed often gave contradictory results on the benefits and problems associated with accelerated vs. nonaccelerated growth rates of Holstein heifers. Importantly, some research showed that, when Holstein heifers were grown at above normal growth rates (accelerated), these heifers may have had impaired mammary gland development that reduced their subsequent milk production. However, not all research obtained results that supported these findings. Clearly, additional research is needed to better identify potential problems in heifer rearing management and to identify management strategies and programs that dairymen can use to rear their heifers. The overall goal is to raise heifers faster so that they are younger at first calving, and that this management practice will not adversely affect subsequent production and/or reproduction.

The transition period represents an enormous metabolic challenge to the dairy cow, especially high yielding cows and thus, is an important focus of current research by scientists and the whole dairy industry. Many abrupt physiological changes occur during this time period that have critical impact on the cows' health, how well nutrients are utilized, how well lactation is initiated, and how much milk is produced during lactation. When the cow fails to meet challenges faced during the transition period, the result often is the occurrence of a range of early postpartum health problems; some fatal and all causing reduced performance and reduced income. Feeding and managing cows and first calf heifers around calving and during early lactation remains one of the big challenges for the dairyman.

Maximizing feed intake increases the supply of energy and glucose from acetate and propionate, and also the supply of amino acids that can be used for milk protein synthesis. Large amounts of these nutrients derived from the diet will reduce the cow's reliance on mobilization of body stores to support milk production and this will result in smaller losses of body condition. Maximizing feed intake also is an important factor in reducing the incidence of metabolic diseases during the early postpartum period. During the close-up period (from 3 wk prepartum to calving), feed intake of cows is affected by their body condition. Emery (1993) found that cows with body condition score (BCS) >3.6 consumed dry matter at 1.5% of body weight, whereas cows with BCS ≤3.6 consumed dry matter at 2% of body weight. Over-conditioned cows also had higher incidence of health disorders postpartum. With reduced feed intake during the transition period that results in reduced availability of needed precursors and energy that drive metabolism, cows turn to lipids stored in adipose tissue to obtain the energy needed. Unfortunately, cows do this poorly during this transition and fat may accumulate in liver setting off a chain of metabolic diseases. Demands for glucose are tremendous at the start of the lactation because the mammary gland has strict dependence on glucose to provide the energy to run the mammary metabolism, to synthesize milk constituents, as a precursor for lactose synthesis and non-essential amino acids used in protein synthesis. Including dietary glucogenic and energy compounds in the close-up diet may help to reduce the need of the cow to mobilize its stored energy and thereby potentially reduce health problems.

These two major factors (accelerated growth of heifers to meet growth goals and the energy demands by first calf heifers and cows during the transition period), that impact mammary gland development and milk production were the impetus to evaluate how different dietary protein and energy concentrations and protein:energy ratios in the diet may affect growth rates and resultant body condition at calving of Holstein heifers which, in turn, will reflect on this health status and milk production. Additionally, we wanted to evaluate possible benefits of supplementing glucose precursors in the transition diets fed to cows around the time of calving that should affect favorably the transition between prepartum and postpartum periods of Holstein cows.

CHAPTER 2

EFFECTS OF FEEDING DIETS WITH DIFFERENT CONCENTRATIONS OF PROTEIN AND ENERGY, PROTEIN:ENERGY RATIOS AND bST ON GROWTH RATES AND FIRST LACTATION OF HOLSTEIN HEIFERS

Introduction

A major goal of every dairy farm operation is to obtain a profit. Dairymen, as well as researchers, are continuously searching for ways to improve management of the dairy operation to reduce costs of production and/or to increase milk production. Methods of raising dairy heifers from weaning to calving often has been overlooked when considering means to improve profitability because it is a non-income generating period in the life of the animal. However, it must be kept in mind that the way in which heifers are raised will affect their performance and production. As Foldager and Sejrsen (1982) indicated, the heifers of today will be the cows of tomorrow and how well they are managed before they enter the herd is one factor affecting profitability.

Raising dairy heifers is the second largest expense that a dairy operation incurs, and it accounts for approximately 20% of the total expenses of the dairy operation (Heinrichs, 1993). One probable reason why the prepubertal growth period of heifers has received limited attention until recent years is that dairymen must invest in feed and labor for up to 2 yr with no immediate positive financial benefits. Nonetheless, researchers have been evaluating ways to reduce costs of raising heifers, which were estimated (Gabler et al., 2000) to range between \$ 896.90 and \$ 1305.00 per heifer. The two greatest components of total costs were feed and labor (60.3 and 13.8%, respectively).

One important option to reduce costs of milk production has been to reduce age at first calving (AFC) of replacement heifers. Recommended growth rates between 10 to 67 wk of age were set at 0.700 to 0.800 kg/d for large dairy breeds, such as Holsteins (NRC 1989). These growth rates allow heifers to achieve BW and physical stature so they could be bred initially at ~350 kg and <15 mo of age. Recently, it has been recommended that replacement heifers should enter the dairy herd at about 22 to 24 mo of age and have a post-calving body weight (BW) of 540 - 570 kg with optimal body condition score (BCS) between 3 and 3.5 (Hoffman, 2002). However, average daily gains in BW (ADG) greater than 0.700 kg/d between 90 and 300 kg of BW have been detrimental to mammary growth (Sejrsen and Purup, 1997) and resulted in decreased milk production. However, reports of negative effects of rapid prepubertal growth rates on mammary growth and milk production have not been consistent when dairy heifers were raised with ADG greater than 0.800 kg/d up to 350 kg of BW. For example, some studies (Sejrsen et al., 1982; Valentin et al., 1987; Waldo et al., 1989) detected reduced milk yields, whereas others did not at ADGs of 0.900 kg/d or greater (Capuco et al., 1988, Van Amburgh and Galton, 1994; Capuco et al., 1995; Pirlo et al., 1997).

Dobos et al. (2000) attributed contradictory results to differences in feeding standards used (Sejersen, 1994) or to diet composition (Kertz et al., 1987). As growth rates of heifers increased, the crude protein (CP) requirements for structural body growth also increased, but at a faster rate than energy requirements (Preston, 1966). Therefore, the protein to energy ratio may be a more appropriate method to evaluate protein requirements of growing heifers (VandeHaar, 1997; VanAmburgh et al., 1998a; Lammers and Heinrichs, 2000).

The rate of mammary epithelial tissue growth during the prepubertal allometric growth phase doubtless is orchestrated by changes in concentrations of growth hormone (GH or ST), estrogen and insulin-like growth factor-1, among others (Purup et al., 1993). Concentrations of ST in plasma of heifers undergoing rapid peripubertal growth are decreased (Sejrsen et al., 1982), whereas injecting bovine somatotropin (bST) increased growth rate of heifers while fat accumulation decreased (McShane et al., 1989; Vestergaard et al., 1993; Radcliff et al., 1997). Additionally, bST injections during the prepubertal period increased mammary cell numbers (Sejrsen et al., 1986; Sandles and Peel, 1987; Radcliff et al., 1997). Rearing prepubertal dairy heifers at accelerated growth rates (>700 g/d) may cause excessive fattening, which in turn may impair mammary secretory tissue development and result in lower MY (Sejrsen and Purup, 1997). Injections of bST reduced the percentage of fat in mammary gland at the same time it increased cell numbers by as much as 47% in one case (Radcliff et al., 1997).

It is clear that there are many factors that can affect mammary growth and subsequent milk production that need to be better evaluated. Therefore, the objectives of this experiment were to evaluate effects of feeding diets with different levels of protein and energy and protein to energy ratios, and the use of bST during a defined growth period (100 d of age to 350 kg of BW) of Holstein heifers to evaluate effects on milk production, BCS, BW, and incidence of disease or health problems around first calving.

Literature Review

Growth Rates of Holstein Heifers and Age at First Calving

It now is recommended that Holstein replacement heifers should calve and enter the milking herd at 22 to 24 mo of age (Heinrichs and Swartz, 1990; Hoffman, 2002). Replacement heifers should be of adequate body size by 22 to 24 mo of age to ensure acceptable first lactation performance and to minimize dystocia (Hoffman and Funk, 1992). Age at puberty can be decreased by feeding higher energy levels, thereby allowing earlier breeding and calving (Gardner et al., 1977), but mammary development and milk yield may be impaired (Sejrsen, 1978).

Foldager et al. (1988), observed onset of puberty in heifers as early as 5 to 6 mo of age and as late as 18 to 20 mo of age, and the variation of BW was between 150 and 400 kg. They reported that less than 5% of the heifers reached puberty before 200 kg BW and less than 10% had first estrus after 300 kg BW. The main source of variation in age at onset of puberty was the level of feeding (see review of Schillo et al., 1992).

To achieve the commonly recommended age (22 to 24 mo) and first post-calving BW (540 - 570 kg), heifers must gain approximately 0.820 kg/d to calve at 24 mo of age. If we wanted these heifers to calve at 22 mo of age or earlier, the growth rate would need to be increased to about 0.890 kg/d (Head et al., 2002). Research has demonstrated that heifers can calve as early as 15 to 16 mo of age (Tucker, 1981). Feeding growing heifers diets that promoted greater growth rates until puberty (about 350 kg BW) resulted in a reduction of AFC. Kertz et al. (1987) reported that growth of replacement Holstein heifers could be accelerated to 1.0 kg ADG from 3 to 12 mo of age without excessive fattening.

A study conducted by Radcliff et al. (1997) evaluated the effects of feeding diets to support ADG of 0.8 kg (standard) or 1.2 kg (accelerated) from 120 d of age until the early luteal phase of the fifth estrous cycle. They found that feeding diets for accelerated growth did not affect the total amount of mammary parenchymal DNA, RNA or the ratio of RNA to DNA. They concluded that diets that contained high energy and high protein that increased growth rates and decreased age at puberty of dairy heifers did not have detrimental effects on mammary development.

Although reducing AFC may result in an economic gain or a reduction in rearing costs, some studies (Gardner et al., 1977; Little and Kay, 1979; Waldo et al., 1998; Van Amburgh et al., 1998b; Radcliff et al., 2000) reported negative or inverse correlations between ADG and MY. Possible reasons why first milk yield was reduced in the younger heifers included decreased mammary development, lack of adequate BW, maturity at calving, and differences in body composition.

Pirlo et al. (2000) reviewed the records of 1,048,942 Italian Holstein-Friesian heifers for possible associations between AFC and milk yield. Their study showed that reduction of AFC below 26 mo of age consistently produced a positive effect on the difference between milk yield returns and rearing costs. Reduction of AFC to 24 and 23 mo seemed to be more profitable than reducing age to 22 mo. They found that the largest positive difference between reduction of rearing costs and milk yield losses was between 23 and 24 mo. Tozer and Heinrichs (2001) reported that reducing AFC from 25 to 24 mo reduced costs 4.3%. Willett et al. (1992) reported that for each additional month of AFC beyond 24 mo, there was an additional $65 in rearing costs. Gardner et al. (1988) examined the records of 433 Holstein cows with AFC of 22.2 and 24.6 mo. Total milk yield through four lactations for heifers with 24.6 mo AFC was 1,221 kg greater than for heifers with 22.2 mo AFC.

Van Amburgh et al. (1994) fed prepubertal Holstein heifers three different diets and achieved AFC of 24.2, 22.0, and 21.0 mo. They reported that milk yield was lower, but did not differ significantly for replacement heifers calving at 22.0 and 21.0 mo as compared to 24.2 mo. Hoffman et al. (1996) evaluated first lactation performance of Holstein heifers fed diets to achieve accelerated or standard ADG during the postpubertal period. Half of the heifers were either target bred or delayed bred. These heifers calved at 20.6, 22.7, 23.6, and 25.6 mo (accelerated growth-target bred, accelerated growth-delayed bred, control-target bred, and control-delayed bread, respectively). They concluded that accelerated postpubertal growth of heifers resulted in earlier calving at similar prepartum BW. However, data for height at withers, pelvic area, and postpartum BW suggested that heifers fed the diets for accelerated growth were smaller. Accelerated postpubertal growth and early calving reduced performance during first lactation. Similar results were found by Radcliff et al. (2000) where heifers fed diets with high energy and high protein had ADG of 1.12 kg compared to 0.77kg for standard fed heifers. Heifers fed the standard diet calved 88 d later than heifers raised on the high energy-high protein diet. Nevertheless, total projected 305-d milk yield was 1,115 kg greater for the standard heifers.

Lammers et al. (1999) hypothesized that estrogen administration during the prepubertal and peripubertal growth periods of heifers would counteract the negative effects of accelerated growth that had been reported. They further hypothesized that estrogen could even increase mammary development and subsequent milk yields. To test their hypotheses they fed Holstein heifers one of two diets during a 20 wk period starting at 4.5 mo of age. Diets were formulated so that ADG of 0.700 kg (standard growth) or 1.0 kg (accelerated growth) would be obtained. One-half of the heifers in each group also received estrogen implants. Heifers grew as expected but the accelerated growth regimen decreased age at puberty by 32 d and decreased first lactation fat-corrected milk yield by 7.1%. Additionally, heifers implanted with estrogen produced 5.2% less fat-corrected milk during first lactation than those heifers not implanted with estrogen. The addition of estrogen in the growth regimen had the opposite effect to that hypothesized.

Abeni et al. (2000) raised Holstein-Friesian heifers using two different diets formulated for ADG of 0.7 and 0.9 kg. These diets were equivalent to 100 and 115% of NRC (1989) recommendations. Half of the heifers were artificially inseminated, after gynecological inspection, at the first estrus observed when they were greater than 370 kg of BW and the remaining heifers at greater than 420 kg BW. They reported ADG of 0.667 and 0.775 kg during the prepubertal period and 0.748 and 0.824 kg during the postpubertal period. These authors found no effects of growth rates on milk production or milk protein percentage, but milk fat was reduced. Additionally, they found that early calving negatively influenced milk production. Possible explanations for the similar responses in milk production of the two groups was that the growth rates were not greatly different from each other and that the highest ADG during the prepubertal period was only 0.775 kg, which was only slightly greater than standard rate.

A fifty percent reduction in mammary development (parenchymal DNA) was observed in heifers raised rapidly during the prepubertal growth phase by Capuco et al. (1995). Increased percentage of fat in these mammary glands seemed to be a consistent finding for heifers raised at accelerated rates (Sejrsen et al., 2000). Perhaps the amount of fat that accumulated in the udder and in the body was just as important as any reduction in mammary parenchymal growth (Head et al., 2002).

There were a number of experiments completed in which the negative effects of feeding level on subsequent milk yield were not observed (Park et al., 1987; Gardner et al., 1988; Head et al., 1991; Van Amburgh et al., 1994). For example, Stelwagen et al. (1992) found no differences in first lactation milk yields when Holstein heifers were raised at ADG of 0.611, 0.737, and 0.903 kg/d between 6 and 16 mo of age. In some cases lack of effect may best be explained by limited differences in the growth rates among treatment groups or because treatment periods evaluated were completely or partially outside of the time period of critical growth (Sejrsen and Purup, 1997). According to Sejrsen and Purup (1997), the available evidence concerning the relationship between prepubertal feeding level, age at puberty onset, mammary growth, and milk yield potential leads to the conclusion that increasing feeding levels above moderate rates results in reduced age at onset of puberty which may lead to reduced mammary growth and reduced milk yield potential. This implies that the time period for the mammary growth to occur was shortened and this resulted in the negative effect. However, in a more recent study, Silva et al. (2002) suggested that growth rates of heifers had little effect on mammary development, but that increased body fatness was a better predictor of impaired mammary development than BW gain.

Protein, Energy and Protein to Energy Ratio

Defining a critical first calving BW is difficult given the influence of genetics and AFC. Keown and Everett (1986) evaluated 305,000 dairy records and found that first lactation milk yields were maximized when heifers weighed

635 kg BW at first calving. Body weight at first calving influenced first lactation milk yield, reproductive performance, and health of first lactation heifers. Heifers that weighed 550 kg post-calving with an AFC between 22 and 24 mo of age showed optimum performance and profitability.

If we assume a calf BW, placenta and fluids weight of 50 kg that is expelled at time of calving, then to achieve critical benchmarks of post-calving BW of 550 kg and AFC of 22 to 24 mo, heifers would need to gain over 0.830 kg/d from birth to weaning. Unfortunately, as described previously, greater rates of growth before puberty may have negative influences on mammary development, growth, and future milk production (Sejrsen and Purup, 1997). Therefore, developing an optimum feeding and management program for heifers requires that we understand the associations among feeding level and amounts of specific nutrients in the diet, the onset of puberty, and peripubertal mammary development (Sejrsen and Purup, 1997). Literature suggests that high rates of gain *per se* are not responsible for some of the negative effects found on milk yield, but the composition of the diet and how it modifies body fat clearly seems to be important in growth and subsequent function of the mammary gland.

Growth and development of mammary cells occurs in distinct phases related to reproductive development (Sinha and Tucker, 1969). The basic structure of the mammary gland is formed during the fetal stage, but the epithelial tissue still is rudimentary at birth (Sejrsen and Purup, 1997). During the first few months after birth, mammary gland growth occurred at the same rate as the rest of the body and this pattern of growth is termed isometric. Beginning between 2 to 3 mo of age the mammary gland starts to grow at a faster rate than the rest of the body and this pattern of growth is termed allometric (Sinha and Tucker, 1969). During this latter phase, there is rapid growth of the fat pad and ducts that branch out into the fat pad and define the structure for later growth. Increased estrogen and the presence of somatotropin induces the ducts to branch and elongate (Akers et al., 2000). This first allometric period ends around the onset of puberty (Sinha and Tucker, 1969; Sejrsen et al., 1982). A second allometric growth phase of mammary gland development occurs during gestation and is provoked by secretions of estrogen and progesterone that promote exponential mammary growth during the last trimester of first pregnancy. Therefore, maximizing mammary parenchymal growth during these allometric growth phases seems to be important to ensure subsequent growth also will be maximal.

Milk production is directly proportional to the number of differentiated mammary epithelial cells. The correlation between total mammary parenchymal DNA and milk production averaged about 0.85. Therefore, understanding these phases of mammary development, their influence on future milk production, and how mammary growth may be affected by the diet is of great importance in heifer management.

From the previous sections, it is clear that diet or plane of nutrition affects mammary development and milk yield, but reasons for the responses often seen and mechanisms that bring about alterations in growth and development of the mammary system still need to be identified completely. Research to date suggests that accelerated rates of gain during postpubertal phase do not impact mammary development, but do influence first-lactation milk yield, and that feeding diets containing higher planes of nutrition during the first allometric phase of mammary growth can have negative impacts on mammary development (Sejrsen and Purup, 1997).

Research by Silva et al. (2002) suggested that growth rates of heifers had little effect on mammary development, and that increased body fatness may be a better predictor of impaired mammary development than BW gain. This and other research results has led to additional research to determine the type and composition of diets that could be fed to increase ADG that would not impair mammary development or subsequent milk production. Furthermore, some studies have evaluated the timing of when specific diets were fed. For example, the negative effects of feeding diets formulated for accelerated growth during the prepubertal and peripubertal periods in the study of Capuco et al. (1995) seemed to be most severe when heifers were fed a corn based diet. However, effects were relatively small when an alfalfa diet with high protein (CP) content was consumed by heifers during the prepubertal and peripubertal periods. This suggested that the type of diet constituents or CP level modified the effects of feeding level on mammary development. VandeHaar (1997) reported a positive relationship (r=0.78) between dietary CP levels and prepubertal mammary development. Interestingly, Kertz et al. (1987) earlier had hypothesized that including a higher level of CP in the diet may reduce or eliminate negative mammary development associated with rapidly grown prepubertal heifers.

Fully understanding protein nutrition in growing ruminants is difficult because it is complex. Nitrogen is a critical nutrient in the ruminant because it is a primary component of protein. The diet that the ruminant actually receives differs from that which is eaten, particularly with regard to carbohydrate and protein. Dietary sources of protein may be degraded in the rumen but this loss is compensated for by microbial protein synthesis. Excessive protein nitrogen in high-protein diets is converted into ammonia, which is absorbed and converted to urea and exported in the urine. This process also utilizes extra energy to detoxify the excess ammonia nitrogen. However, protein in the diet is supplemented by microbial synthesis using endogenous recycled urea, and this can lead to more protein being available in the intestines than was fed to the animal (Van Soest, 1994). Bacterial protein is utilized by the ruminant as a source of amino acids and peptides, which, in addition to dietary protein that escaped rumen degradation, also is used for accretion of tissue or synthesis of milk protein in the lactating animal.

The type or source of protein fed to growing heifers has been evaluated and also has yielded inconsistent conclusions. Responses of growing heifers to rumen undegradable protein (RUP) has been inconsistent and appeared to vary among protein sources fed. For example, Tomlinson et al. (1997) reported improved feed efficiency as RUP percentage of CP in diets increased from 31 to 55%, when blood meal was the source of RUP. Thonney and Hogue (1986) reported improved feed efficiency, but not improved ADG, when cottonseed meal was replaced with fish meal in the diet of growing Holstein steers. Bethard et al. (1997) fed diets with either 30 or 50% RUP. They reported that heifers fed the high RUP diets used feed nutrients more efficiently, but dietary treatment from 6 to 13 mo of age did not affect BW at calving, AFC, or ADG from birth to calving. Other researchers (Mantysaari et al., 1989) found no responses in ADG or feed efficiency when higher RUP diets were fed. Van Amburgh et al. (1998b) evaluated diets containing different amounts of RUP and the feeding level on first lactation milk production. No differences were detected that were attributed to different levels of RUP in the diet. Swartz et al. (1991) reported on the effects of feeding different RUP levels in diets during two age periods. They measured DMI, growth, feed efficiency, and carcass composition of the growing heifers. Growth period one was from 1 to 12 wk of age (33, 37, and 46% RUP), and growth period two was from 14 to 25 wk of age (30, 34, and 38% RUP). They detected no differences across treatments or between periods except for increased feed efficiency as RUP level fed increased during the second period. In general, feeding greater amounts of RUP has not resulted in significant or consistent improvement in heifer growth or feed efficiency. Clearly, additional research is needed to determine the effects of RUP percentage and time or duration of feeding. Only after accumulating additional information can sound recommendations on level of RUP be made and incorporated into heifer feeding strategies.

Dobos et al. (2000) fed three diets with different CP (14.2, 18.3, and 18.2%) and RUP amounts (27.0, 13.3, and 26.7% of total CP) to heifers from 5 to 10 mo of age. They reported that heifers fed the high-CP diets had less mammary fat tissue and a lower ratio of fat to secretory tissue compared with those on the low-CP diet. Additionally, age and BW at calving were not influenced by either dietary CP or RUP concentrations. Importantly, they reported that first lactation milk, fat, and protein yields were not influenced by prepubertal dietary CP concentrations. In a review on RUP prepared by Santos et al. (1998), the authors concluded that, in lactating cows, when a high quality RUP source replaced a low quality source there was an increase in milk yield. This seemed to confirm that dietary protein quality was the major factor determining whether RUP improved lactational performance. They indicated that it is necessary to consider independently the adequacy of RUP and RDP in diets of lactating cows. Importantly, they considered it to be illogical to increase RUP at the expense of RDP unless RDP is excessive.

While some data (Kertz et al., 1987; Daccarett et al., 1993) suggested benefits arose when dietary CP fed to dairy replacement heifers was increased, excessive feeding of CP is problematic. Excessive feeding of CP to dairy heifers resulted in increased fecal and urinary-N excretion (Wilkerson et al., 1993; Gabler and Henrichs, 2003). Furthermore, feeding excessive protein to postpubertal dairy replacement dairy heifers presented the greatest potential for detrimental N excretion into the environment because gross N-excretion was positively correlated to BW (Wilkerson et al., 1993). Gabler and Henrichs

(2003) reported that diets with 20% CP were economically and environmentally costly due to high N excretion and no difference in total apparent digestibility within the digestive tract was detected compared to diets that contained 18% CP or less.

Radcliff et al. (1997) fed prepubertal heifers diets that contained either 16.3% CP formulated for 0.800 kg ADG, or 19.4% CP formulated for 1.2 kg ADG. They reported that the high CP diet did not affect total amount of parenchymal DNA, RNA or the ratio of RNA to DNA in mammary tissue. They concluded that high protein diets increased growth rates and reduced age at puberty and that no negative effect was observed on prepubertal mammary development. In a later but similar study, Radcliff et al. (2000) reported that heifers fed a standard diet (17.5% CP) reached puberty 90 d later than heifers fed a high protein diet (19.3%), but postpartum BW, BCS, and calving ease scores of heifers fed the standard diet did not differ from those fed the high protein diet. Additionally, heifers fed the standard diet produced 14% more milk than heifers fed the high CP diet. Thus, from these data it appeared that the diet containing 17.5% protein provided sufficient protein and had no negative effects compared to those fed about 10% more protein.

Veira et al. (1980) reported results of increased dietary CP levels fed to heifers that had been weaned from milk replacer at 4 wk of age. A Latin square design was used for this growth trial to evaluate diets that contained either 9.9, 12.0 14.2, or 16.2% CP. Nitrogen retention increased with dietary

CP levels in a linear fashion and DMI and ADG increased as CP increased in the diet fed.

The NRC (2001) indicated that the amount of protein consumed daily to achieve target growth rates in growing heifers is the sum of 1) rumen degradable protein (RDP) required for microbial growth that can be achieved given an adequate amount of rumminally degraded carbohydrates, and 2) RUP required to supplement the microbial protein produced to support the energy allowable average daily gain. Preston (1966) reported that protein requirements of growing ruminants have little meaning unless energy requirements have been satisfied. Kertz et al. (1987) indicated that the increased energy content of diets fed in their research resulted in increased rates of gain but acknowledged that dietary CP levels less than 17% may be limiting in diets formulated with enough energy to achieve 0.800 kg/d ADG.

The NRC (2001) indicated that the amount of energy that is required for growth is calculated from the net energy that is deposited. Growth rates of heifers depends on the net energy available after maintenance requirements have been met. Therefore, as growth rate increases for a given BW, the net energy for gain increases. When energy intake increased above that required for maintenance, the rate of protein deposition became limiting and this resulted in excess energy being deposited as fat. As mentioned previously, excess energy deposited as fat in depot reserves of the heifer and in the udder may impair milk production. Additionally, Akers et al. (2000) reported that excess energy resulted in increased IGF-I binding to IGF-I binding protein-3 which prevented access to IGF binding protein-I and corresponding mitogenic proliferation of mammary secretory cells.

Increased levels of energy in the diet of prepubertal heifers resulted in greater ADG and body size. Because onset of puberty is associated with BW, greater growth rates corresponded to earlier ages at puberty. However, if energy intake was in excess and protein became limiting, the higher growth rates may result in excessive fat deposition.

A review by Russell and Hespell (1981) emphasized the need for synchronous feeding of ruminally degradable carbohydrates and protein to increase nutrient utilization and performance. Later, Hoover and Stokes (1991) explained that microbial protein yield in the rumen depended largely on the availability of dietary carbohydrates and protein, thus synchronization of the rate of carbohydrate fermentation and protein degradation was a viable way to optimize microbial growth.

As growth rates of heifers increases, the crude protein (CP) requirements for structural body growth also increases, but at a faster rate than energy requirements (Preston, 1966). Therefore, the protein to energy ratio may be a more appropriate way to evaluate protein requirements (Lammers and Heinrichs 2000). The NRC (1989) determines CP requirements based on BW, ADG, protein digestibility, DMI, and energy density of the diet. However, according to Lammers and Heinrichs (2000), to increase growth rates above the NRC (1989) recommendations of 0.700 kg/d requires that a more energy dense diet be formulated and fed than that recommended by NRC (1989).

Numerous studies (Kertz et al., 1987; Radcliff et al., 1997; Van Amburgh et al., 1998a) have evaluated dietary protein and energy contents of diets on Holstein prepubertal heifer growth. Yet, few have evaluated protein and energy together or the association of the ratio of the two components (CP:ME). Schurman and Kesler (1974) evaluated CP:ME ratios (49.3, 52.2, and 89.7 g of CP per Mcal of ME) in ruminating dairy calves from 74 to 142 kg of BW. They reported superior growth rates and feed efficiency with the two lowest CP:ME ratios in the diets fed. Lammers and Henrichs (2000) studied the effects of diets differing in CP:ME ratios on growth of prepubertal heifers from 200 to 341 kg BW. These researchers set daily energy intake at 16 Mcal of ME with the amount of dietary CP set to provide desired ratios of CP:ME of 46, 54, and 61 g of CP per Mcal of ME. Dry matter was restricted at 2.45% BW of the heifers. Results obtained showed that heifers consuming the diet with highest CP:ME ratio (60.9) had the greatest rate of BW gain, feed efficiency, structural growth, and growth of mammary ductal development, as obtained by indirect measurements.

In another study by the Pennsylvania research group, 60 Holstein heifers 124.5 d of age and weighing 125 kg BW on average were fed diets with different CP:ME ratios for a period of 20 wk (Gabler and Heinrichs, 2003). The CP:ME ratios were 48.3, 59.1, 67.5, and 76.5 g CP per Mcal of ME. The CP:ME ratios were altered by adjusting the concentration of CP (12.0, 15.2, 17.4, and 19.7% CP) with similar amounts of ME (2.6 Mcal/kg DM) across all treatment rations. Authors reported similar ADG across all treatments, but feed efficiency increased and plasma levels of urea N also increased as the CP:ME increased.

Studies by Kertz et al. (1987) and Dacarett et al. (1993) suggested that if adequate protein is provided relative to energy, ADG may be greater than 0.8 kg during the prepubertal phase without over-conditioning the heifers. Recommendations from the NRC (1989) require that concentrations of RUP in the diet be greater than 50% of the CP intake for heifers that weigh less than 250 kg. This implied that light weight heifers produced considerably less microbial protein than needed to meet overall tissue protein requirements. However, performance and feed efficiency have not been consistently improved in young, light-weight heifers when supplemental RUP was fed.

Many studies have shown that feeding prepubertal heifers diets formulated to promote accelerated growth (> 0.800 kg/d) showed decreased mammary development and subsequent milk yield often was reduced. The magnitude of the reduction varied from study to study, perhaps due to variations in the protein to energy ratio. VandeHaar (1997) examined the relationship between mammary development or milk yield and the protein to energy ratio from eleven studies in which ADG of heifers during prepubertal growth period exceeded 0.900 kg/d. The CP:ME ratio in diets fed in these studies varied from 43 to 83 g CP/Mcal. Fifty-one percent of the variation in mammary parenchymal response and 78% of the variation in milk yield response to rapid growth rates were attributed to the CP:ME ratio. Whitlock et al. (2002) indicated that one limitation of this literature analysis was that protein was evaluated as CP rather than as metabolizable protein (MP). When MP was estimated and MP:ME calculated for 4 of the 11 studies, the MP:ME ratio accounted for 88% of the variation in parenchymal DNA response to high-energy intake across these studies.

Whitlock et al. (2002) fed Holstein heifers diets that contained 2.85 Mcal/kg and MP at low (37 g/Mcal of MP), standard (41 g/Mcal of MP) and high (44 g/Mcal of MP) concentrations from 3.5 mo of age until puberty. The ADG for heifers fed the low, standard, and high protein were 1.13, 1.17, and 1.18 kg/d, respectively. They found that dietary protein did not affect age or BW of heifers at puberty or slaughter, withers height gain, or carcass composition. Average mammary parenchymal DNA did not differ significantly but tended to be greater with increased diet MP:ME ratios (595, 619, and 670 mg DNA/100kg of BW for low, standard and high). They concluded that dietary protein did not have a major effect on mammary development of rapidly grown prepubertal heifers, provided the diet contained adequate protein for normal growth. The authors (Whitlock et al., 2002) suggested that feeding low-protein diets increased the risk of impaired mammary development when heifers were fed for rapid growth and attained puberty early and that the NRC (2001) guidelines for protein relative to energy seemed to be adequate for optimal mammary development.

Bovine Somatotropin

The rearing of replacement dairy heifers contributes greatly to the total costs associated with milk production. Dairy producers must make large investments for feed, housing, labor and maintenance of good health, but no income is received until heifers are either sold or they enter the milking herd. Increasing prepubertal growth rates decreases the AFC which may result in reduced costs. However, as described in previous sections, this often results in lower milk yield. The decrease in milk production that is associated with feeding high-energy diets during the prepubertal period have been attributed to reduced growth of mammary parenchyma and/or a concurrent increased deposition of mammary adipose tissue (Swanson, 1960). The use of bovine somatotropin (bST) increased growth rates and decreased carcass fat accumulation in growing heifers (McShane, et al., 1989; Moseley et al., 1992), and increased mammary parenchymal tissue and decreased mammary adipose tissue contents.

An excellent review on bST was published by Bauman (1992). A decade has passed since that review and many additional studies have been conducted using bST. However, most research has been with lactating cows and the use of bST in growing heifers is much more limited.

Some early studies on use of bST in heifer growth detected no effects on conception rates, calving difficulty or subsequent first lactation milk yield when heifers were injected from 7 mo of age to puberty (Murphy et al., 1991), for five mo after reaching 295 kg BW (Grings et al., 1990), during pregnancy (Stelwagen et al., 1992) or from 30 d postpartum through the end of the lactation (Morbeck et al., 1991). No effects of bST on ADG were detected in these studies except by Grings et al. (1990); their injected animals gained an additional 0.180 kg/d. However, when bST was injected during lactation various other groups (Eppard et al., 1987; McShane, et al., 1989; Dahl et al., 1991; Eppard et al., 1991; Moseley et al., 1992; Bauman and Vernon, 1993) have reported 19 to 40% increase in milk yield. Supplementation of bST not only caused increased milk yield, but production persistency also was improved.

Indeed, as indicated it is well documented that somatotropin administration to cattle during lactation stimulates milk production. This effect was indirect in that somatotropin stimulated secretion of IGF-1 from the liver, which in turn mediated many of the galactopoietic effects of ST during lactation. Somatotropin also acted as a mammogeinic hormone and was one factor that stimulated mammary growth at all stages of development. Direct effects of ST on mammary tissue would require the presence of specific ST receptors on mammary cells. It was hypothesized that ST would stimulate prepubertal mammary development even though some research reported that no specific ST receptors were found in mammary tissues (Purup et al., 1995). Although this remains a point of controversy, there is evidence that ST receptors can be found in mammary epithelial or stromal cells in various species. Somatotropin may act on ruminant mammary tissue by stimulating stromal production of IGF-1, which is mitogenic, and IGF-1 binding proteins (Sejrsen et al., 1986). Binding of IGF-1 by IGF-1 binding proteins would stimulate mammary cell proliferation.

Mammary ductal development in prepubertal heifers is modulated by localized synthesis of IGF-1 and IGF-1 binding proteins (Weber et al., 2000). The highest level of IGF-1 expression in mammary tissue occurs in the fat pad and is greatest during the prepubertal allometric growth phase and during late pregnancy. Mammary expression of IGF-1 is regulated by ST, estrogen and a positive feedback stimulation from proliferating epithelial cells. Insulin-like growth factor-1 activity is highly regulated by mammary tissue localized IGF-3 binding protein (Weber et al., 1999). Weber et al. (2000) showed that elevated levels of IGF binding protein-3 was exhibited by prepubertal heifers fed high planes of nutrition. Research by Akers et al. (2000) showed that IGF binding protein-3 could inhibit mammary DNA synthesis (33%) by binding localized IGF-1 in *in vitro* cultures, similar to results reported by Weber et al. (1999). This latter group reported that mitogenic activity in mammary extracts was inhibited by 35, 50, and 82% by IGF-1 binding protein-3 when it was added to the medium at 1, 2 and 3 times the molar IGF-1 concentration in the extract. Additionally, they reported a 26% reduction in DNA synthesis.

Radcliff et al. (1997) injected Holstein heifers daily with 25 µg bST/kg of BW from 120 d of age until the early luteal phase of the fifth estrous cycle. They reported that injections of bST increased ADG, skeletal size and mammary development. The injections of bST increased mammary parenchymal DNA, RNA, and the ratio of RNA to DNA. In a later study, Radcliff et al. (2000) fed Holstein heifers diets formulated for ADG of 0.80 (standard) or 1.20 kg/d (high) in conjunction with daily bST injections (high-b;

25 µg bST/kg of BW). Heifers were treated from 135 kg BW until they became pregnant. Heifers on the high and high-b treatments were approximately 90 d younger than standard heifers at first insemination and first calving. Furthermore, they reported no differences between treatments for postpartum BW, BCS, and calving ease scores. Heifers fed the high diet (no bST) produced numerically less milk (14%) during the first lactation, but the difference was not significant.

Garcia Gavidia (1998) fed Holstein heifers diets to support ADG of 0.680 kg or 0.900 kg/d, and supplemental bST. They found that bST stimulated growth rates irrespective of whether heifers were fed for normal or rapid growth rates. They suggested that bST injections improved growth rates by increasing feed efficiency and nutrient distribution to priority organs. Thus, bST-injected heifers could have been bred earlier and calved at younger ages, which agreed with findings of Radcliff et al. (2000).

Materials and Methods

Experimental Design

Holstein heifers (n=121) were fed one of four diets that contained different percentages and ratios of protein and energy during prepubertal and peripubertal growth periods. Diets were fed from 125.2 ± 3 d of age until they were 341 kg BW. Diets were formulated to contain either 14% (L) or 18% (H) crude protein (CP) and energy concentration at 100% (L) or 110% (H) of NRC (1989) requirements to give four protein-energy diet groups: LL (n=25), LH (n=21), HL (n=24), and HH (n=18). The four diets contained 53, 50, 67, and

63 g of CP/Mcal ME, respectively (Table 1). Diets were formulated for average daily gains (ADG) of 0.80 kg/d (LL and LH) or 1.08 kg/d (HL and HH). At body weights greater than 341 kg all heifers were fed a diet to support recommended growth rates (NRC, 1989) until they were bred and confirmed pregnant.

Only eighty-eight heifers, from the larger group of 121 heifers, completed 150 d in milk (DIM) and were used to evaluate the effects of diet, season of calving (SEA 1, October-March; SEA 2, May-September), and bST (POSILAC®, 500 mg bST/1.4 ml) on calving variables, milk yield (MY), and postpartum BW and BCS.

One-half of the heifers in each group also was injected with bST at 14 d intervals. Injections were started at 125.2 ± 3 d of age and were discontinued when the heifers reached 341 kg BW, irrespective of age. The administered dose was adjusted to ensure at least 28 µg of bST/kg BW/d (0.2 mL to 181 kg BW, 0.3 mL from 182-273 kg BW, and 0.4 mL above 273 kg BW).

Measurements and Statistical Analysis

Beginning at 125.2 ± 3 d of age, BW and height at withers were measured weekly through 341 kg BW. Measurements of height at withers was discontinued at this point but BW and BCS measurements were taken at breeding, at mid-pregnancy and at first calving. During the postpartum period, BW and BCS measurements were taken weekly through 28 DIM and thereafter biweekly through 150 DIM. These measures were taken between 0600 and 1100 h. Daily milk production was recorded from calving through 150 DIM.

Table 1. Percentage of constituents and composition of the four total mixed rations (TMRs) fed to growing dairy heifers, dry matter basis.

Composition	LL[1]	LH[1]	HL[1]	HH[1]
Ingredients (%)				
Corn Silage[2]	40.5	30.0	40.5	30.0
Alfalfa Hay[3]	15.0	11.2	15.0	11.2
Ground Corn	16.4	29.5	10.0	20.6
Citrus Pulp	15.7	16.0	7.5	11.0
Soybean Meal	6.2	7.1	16.8	18.0
Calf Grower[4]	6.0	6.0	10.0	9.0
Trace Mineral salt[5]	0.2	0.2	0.2	0.2
Nutrients				
DM (%)	62.7	69.2	63.2	69.7
ME (Mcal/kg)	2.75	2.86	2.75	2.86
CP (%)	14.40	14.27	18.37	18.03
MP (%)[6]	11.28	12.05	12.69	13.37
RUP (%)[7]	34.0	37.5	33.0	36.0
RDP (%)[7]	66.0	62.5	67.0	64.0
Ca (%)	1.46	1.40	0.88	0.82
P (%)	0.35	0.35	0.41	0.40
g CP:ME Mcal	53.0	50.0	67.0	63.0
g MP:ME Mcal	41.0	42.1	46.1	46.7

[1] LL (Low protein-Low energy), LH (Low protein-High energy), HL (High protein-Low energy), HH (High protein-High energy).

[2] Corn silage on average contained 34.6% DM, 52.1% NDF, 34.2 %ADF, and 11.3% CP, DM basis.

[3] Alfalfa hay on average contained 94% DM, 41.6% NDF, 34.2% ADF and 18.4 CP, DM basis

[4] Lakeland Cash Feed Company, Lakeland, FL. Contained 88.7% DM, 10.5% NDF, 21.2% ADF and 19% CP, DM basis.

[5] Flint River Mills, Bainbridge, GA. Contained 96.5% NaCl, 100 ppm Co, 500 ppm Cu, 70 ppm I, 3,500 ppm Fe, 2,500 ppm Mn and 7,500 ppm Zn, DM basis.

[6] Metabolizable protein (%)= 0.64 x microbial protein + 0.8 x %RUP of CP x %CP, where microbial protein = 3.8 x Mcal of ME/kg DM (Whitlock et al., 2002).

[7] Estimated from NRC (1989).

Records of number of inseminations, days open, incidence of diseases, calving difficulties and weight and sex of calves born were obtained from daily health records of the UF/IFAS Dairy Research Unit and were used for retrospective analyses.

Data were analyzed by PROC GLM least squares analyses of variance (SAS, 1993) and PROC MIXED methods of SAS (Littell et al., 2000). Regression and correlation analyses were used to evaluate associations among measures and responses of heifers. The model used included main effects of TRT, SEA, bST, their two- or three-way interactions, and the residual error term. Cow nested within the interaction of TRT by SEA by bST was used as the random variable. Differences of least squares means (PDIFF) obtained form GLM and MIXED methods of SAS were used to determine differences between means. Milk production data were adjusted by covariance for BW, and prepubertal analyses by initial age (IAGE) as appropriate. The statistical model used was:

$$Y = \mu + D_i + S_j + B_k + DS_{ij} + DB_{ik} + SB_{jk} + DSB_{ijk} + \epsilon_{ijk}$$

where:

Y= is the dependent variable,

μ= is the overall mean,

D_i= is the effect diet i,

S_j= is the effect of season j,

B_k= is the effect bST treatment k,

ϵ_{ijk}= is the residual error.

Results and Discussion

Of the initial 121 heifers assigned to the growth phase of this study, only 88 completed 150 DIM (~73%) and were used in the statistical analyses. Summary of the distribution and reasons for heifer losses from experiment are in table 2. Most of the heifers lost were due to reproductive failure and never became pregnant (68.75%), whereas only two heifers aborted (6.25%) and two had liver-related problems (6.25%). The remaining 18.75% of heifers lost were removed from experiment due to injuries. Number of heifers removed from trial was numerically greater for diets high in energy compared to low energy (28.13% for LH and 34.37% for HH vs. 18.75% loss for LL and 18.75% HL). Number of heifers removed due to reproductive problems was numerically greater for HH (n=8) and lowest (n=2) for LL. Of the 32 heifers removed, 16 were treated with bST and 16 were not. Of the bST- treated heifers, the numbers lost from each diet group were 4, 5, 3, and 4 for diets LL, LH, HL, and HH, respectively. Number of animals were to few to evaluate data statistically. Ingredient and nutrient composition of the four diets fed during the growth period are in table 1. Metabolizable CP and the calculated RDP, as a percentage of dietary CP, were similar across the four diets. Grams of MP per Mcal of ME calculated for the four diets were 41.0 , 42.1, 46.1 and 46.7 for LL, LH, HL, and HH, respectively. On average and across diets, heifers began consuming the treatment diets at 125.7 ± 3 d of age and 118.3 ±15.5 kg of BW. Changes in BW and increase in height, expressed as ADG and average high increase (AHI), were evaluated using Proc Mixed and the mathematical model

Table 2. Summary of causes for heifer removal from experiment

CAUSES	LL [1]	LH [1]	HL [1]	HH [1]
Left displaced abomasum	---	---	---	1
Reproductive Failure [2]	2	7	5	8
Aborted	1	1	---	---
Died (calving associated)	---	1	---	---
Mastitis	1	---	---	---
Liver-related problems	1	---	---	1
Back injury	---	---	---	1
Bad legs	---	---	1	---
Sold	1	---	---	---
Total heifers removed from experiment, by treatment	6	9	6	11
Percentage lost	18.8	28.1	18.8	34.4
bST-treated heifers removed	4	5	3	4

[1] LL=low protein-low energy, LH=low protein-high energy, HL=high protein-low energy, HH=high protein-high energy.

[2] Includes tract problems, none breeders, dystocia.

described in table 3 during the time period heifers were consuming treatment diets. Average daily gains (Table 3) up to 341 kg, as determined by changes in BW, differed ($P=0.0433$) among treatments (0.899, 0.977, 1.035, and 1.007 kg/d for LL, LH, HL, and HH, respectively). Heifers fed the LL diet had lower ADG compared to LH, HL and HH treatments ($P \leq 0.0605$) but no differences among these three diets were detected ($P>0.1280$).

Least squares means differences (PDIFF) analyses showed that heifers fed the two lower protein diets had greater ADGs than expected and they differed from each other (LL=0.899 and LH=0.977kg/d; $P=0.0605$), whereas they were essentially as expected but not different for groups fed the two high protein diets (HL=1.035 and HH=1.007 kg/d; $P=0.4307$). A similar tendency due to protein in diet was observed by Van Amburgh et al. (1998b) in studies that evaluated the NRC (1989) and Cornell Net Carbohydrate and Protein systems for predicting the requirements for growth of Holstein heifers. They reported that ADG of heifers fed diets formulated for ADG of 0.600 and 0.800 kg were higher than target growth rates, and that heifers fed diets formulated for ADG of 1.0 kg were lower than target growth rates. They concluded that the NRC (1989) tended to underestimate RDP and overestimate RUP requirements. Moreover, they concluded that the NRC equations for estimation of energy and protein requirements did not adequately reflect the biology of growth in dairy replacement heifers and required refinement. This explains, in part, why heifers during the current study may not have performed as expected. Another reason, although less likely to have caused effects

Table 3. Least squares means of growth variables of Holstein heifers fed diets with different amounts and ratios of crude protein and energy (CP:ME; g/Mcal) during the prepubertal period.

Variable[1]	LL[2]	LH[2]	HL[2]	HH[2]	±MSE[2]
IAGE (d)	128.7	126.4	122.9	124.8	1.9
DOT (d)	248.1	246.4	217.1	238.7	7.5
FAGE (d)	376.8	372.8	340.0	363.5	6.4
IHGT	96.6	91.4	95.3	90.8	0.9
FHGT	124.1	123.0	123.6	124.5	0.7
THI (cm/d)	0.115	0.135	0.129	0.135	0.007
IBW (kg)	133.5^c	108.8^a	123.2^b	106.4^a	3.4
FBW (kg)	341.4	346.7	344.2	345.8	1.2
ADG (kg)	0.899^a	0.977^b	1.035^b	1.007^b	0.02

[1] IAGE= initial age, DOT=days on treatment, IHGT=initial height at withers, FHGT= final height, THI=total height increase, IBW=initial body weight, FBW=final body weight, ADG=average daily gain from 125.2 ± 3 d through 341 kg BW.

[2] LL=low protein-low energy, LH=low protein-high energy, HL=high protein-low energy, HH=high protein-high energy, MSE=pooled mean standard error.

[abc] Means with different letters within rows differed (P<0.1;PDIFF).

seen, could be that the actual specific nutrient content of the diets as fed differed slightly from that computed for formulation. This would imply that bias in the diet formulation always was in one direction which would be highly unlikely. Indeed, it is much more likely that microbial protein synthesis was overestimated.

Overall, age at which heifers reached 341 kg BW (FAGE) was similar across the four diet groups (P= 0.1483), but diet fed did affect ADG (P=0.0433), whereas height at withers at 341 kg BW was not affected by diet (P=0.6594). Although this statement appears to be contradictory, these results may be explained by the different IAGE, IBW and FBW across treatments. Least squares means for days to reach final BW, indicated by days on trial (DOT), are in tables 3 and 4. Heifers fed the high protein diets that differed in energy content had similar ADG (1.035 and 1.007kg/d, HL and HH, respectively). Although no significant TRT effect was detected, heifers fed the high protein low energy diet (HL) reached 341 kg BW 21.7 d earlier compared to heifers on HH, 31.1 d earlier compared to LL (P=0.0667), and 29.4 d earlier compared to LH (P=0.0541). On average, heifers fed the two high protein diets (HH and HL diets) reached 341 kg BW earlier than those fed the lower protein diets (LL and LH diets; 247.3 vs. 227.9 d of age for H protein and L protein groups). When compared by energy level in the diets, the L energy diet groups reached 341 kg BW 10.0 d earlier than the H energy diet groups (242.5 vs. 232.5 d of age).

Table 4. Least squares means of growth variables of Holstein heifers injected or not injected with bST during the prepubertal period and during two seasons.

	bST group			Season assigned to trial		
Variable[1]	bST[2]	CON[2]	±MSE[2]	SEA 1[2]	SEA 2[2]	±MSE[2]
IAGE (d)	124.4	127.0	1.3	127.3	124.1	1.4
DOT (d)	234.1	241.0	5.2	238.2	237.0	5.3
FAGE (d)	358.5	368.0	6.1	365.5	361.1	6.1
IHGT (cm)	92.9	94.2	0.6	92.9	94.2	0.6
FHGT (cm)	123.7	123.9	0.6	124.0	123.6	0.6
THI (cm/d)	0.130	0.126	0.005	0.130	0.127	0.005
IBW (kg)	114.7	121.3	2.3	115.9	120.1	2.4
FBW(kg)	345.6	343.4	0.8	344.6	344.4	0.8
ADG (kg/d)	0.998	0.960	0.13	0.985	0.974	0.014

[1] IAGE= initial age, DOT= days on treatment, IHGT= initial height at withers, FHGT= final height at withers, THI= total height increase, IBW= initial body weight, FBW= final body weight, ADG=average daily gain from 125.2 ± 3 d through 341 kg BW.

[2] bST= bST treatment, CON= control (no bST), SEA 1= season 1 (October-March), SEA 2= season 2 (May-September), MSE= pooled mean standard error.

Apparent inconsistency in diet consumption and actual growth rates probably reflects the actual amount of diet that the groups of heifers consumed, as well as the concentration of protein or energy in the diets formulated. Overall, average DMI estimated from daily amounts fed minus daily weighback for the four diets were 7.84, 7.39, 8.37 and 7.6 ± 0.07 kg/d ($P<0.01$) for LL, LH, HL and HH, respectively . Based upon estimated intake of the groups, daily CP intake and ME intake for LL, LH, HL, and HH were 1.15, 1.07, 1.66, 1.46 ±0.01 kg ($P<0.01$) CP, and 21.99, 21.48, 24.98 and 23.31 ±0.21 Mcal ($P<0.01$), respectively. These are only rough estimates because of the way in which daily feed intakes were estimated. Because of this they can not be directly compared to results of other research where individual animal intakes were obtained daily. However, they can be used to compare estimated intakes and efficiencies within this study.

With previously mentioned limitations, feed efficiency (FE; kg DMI/kg of BW gain) for the four diets were 9.18, 7.78, 8.48, and 7.76 kg DMI/kg of BW gain for diets LL, LH, HL, and HH, respectively. These efficiency values were greater (less efficient) than those reported by Lammers and Heinrichs (2000) and Whitlock et al. (2002). Lammers and Heinrichs (2000) reported FE of 6.38, 6.28 and 5.98 for diets with CP:ME ratios of 46, 54 and 61 g CP/Mcal ME, respectively. Whitlock et al. (2002) reported even better FE values. These researchers fed Holstein heifers diets that contained 48, 57 and 66 g CP/Mcal ME which resulted on FE of 5.48, 5.27 and 5.18 kg DMI/kg BW. Both research groups fed diets with similar CP:ME ratios and reported better efficiencies as the rate of CP:ME increased. Results from the current experiment showed no linear pattern which contrasts results of Lammers and Heinrichs (2000) and Whitlock et al. (2002) even though the CP:ME ratios were similar. Nonetheless, heifers fed high protein diets were more efficient, they had numerically lower FE values (8.12 kg DMI/Kg of BW) compared to low protein diets (8.48 kg DMI/Kg of BW). Additionally, FE improved within protein groups as energy was increased (8.48 vs. 7.76 DMI/kg of BW gain for HL and HH, respectively). Possible explanations for the discrepancy in results likely was, in part, due to diet composition and also due to the imprecise DMI measurement procedure. Weather conditions (ambient temperature) also might have affected performance of heifers in the current study. It is well known that dairy cattle perform better during cooler temperatures. Bucklin et al. (1992) indicated that optimal performance of heifers with ADG between 0.8 and 1.5 kg/d was achieved when ambient temperatures were between about -10 and 13°C.

Total height increase (THI) at withers from age assigned to experiment to 341 kg BW was not affected by diet, bST treatment, SEA, or by the two- or three-way interactions. Heifers fed the low protein diets gained less height than those fed the high protein diets. Within protein groups, increased energy levels resulted in slightly greater numerical height gains at withers, although differences were not significant (Table 3). Heifers not injected with bST gained slightly less height through 341 kg BW (0.126 vs. 0.130 cm/d) than did injected heifers. Although bST injections had no significant effects on any of the prepubertal measures evaluated, ADG of heifers injected with bST was numerically 3.96% greater (P=0.6226) compared to heifers that were not injected (Table 4).

No significant differences were detected in number of inseminations, calving age, calving BCS or calving BW due to diet or bST injections (Tables 5-8). On the other hand, heifers that calved during the cooler season (SEA 1) had lower BCS (P=0.0436) and weighed less at calving (P=0.0172; Table 6) probably because these heifers had passes through the warmer season before calving. No significant two- or three-way interactions were detected .

Accelerated prepubertal growth rates of heifers resulted in a decrease in subsequent milk production in some studies (Foldager and Sejrsen,1991; Lammers et al., 1999), whereas Waldo et al. (1998) reported no significant effects on milk production. Capuco et al. (1995) reported a 48% decrease in mammary parenchymal DNA with accelerated prepubertal growth rates, however, this effect did not cause a decline in subsequent milk production in heifers reared the same. VandeHaar (1997) indicated that the protein to energy ratio accounted for 51% of the variation in mammary development and 78% of the variation in milk yield responses to rapid growth rates. He suggested that increased quantities of dietary protein might be needed for optimal mammary development. In the current study, MY through 150 DIM did not differ (P=0.9263) among the four diet groups (Figure 1). Mean 150-d MY of heifers in the four diet groups were 30.0 (LL), 30.1 (LH), 28.6 (HL), and 29.9 (HH) kg/d (Table 7). Relative to CP in the diet, similar results were

Table 5. Least squares means and MSE for calving variables of Holstein heifers fed diets with different amounts and ratios of crude protein and energy (CP:ME; g/Mcal) during the prepubertal period.

	LL[1]	LH[1]	HL[1]	HH[1]	±MSE
Age at calving (mo)	24.0	23.8	22.8	23.7	0.5
Number of inseminations	2.4	2.5	2.1	1.8	0.4
Calving Weight (kg)[2]	540.2	522.7	507.6	515.0	12.3
BCS at Calving	3.5	3.4	3.4	3.6	0.1
Gestation length (d)	276.0	265.0	268.8	270.5	5.2

[1] LL= low protein-low energy, LH=low protein-high energy, HL= high protein-low energy, HH= high protein-high energy, MSE= pooled mean standard error.
[2] Within 24 h after calving.

Table 6. Least squares means and MSE for calving variables of Holstein heifers injected or not injected with bST during the prepubertal period and during two seasons.

	bST group			Season assigned to trial		
	bST[1]	CON[1]	±MSE[1]	SEA 1[1]	SEA 2[1]	±MSE[1]
Age at calving (mo)	23.4	23.7	0.32	23.5	23.6	0.33
Number of inseminations	2.1	2.2	0.2	1.9	2.4	0.3
Calving Weight (kg)[2]	513.6	528.8	8.3	506.1[a]	531.2[b]	8.7
Calving BCS	3.5	3.5	0.1	3.4	3.6	0.1
Gestation length (d)	271.9	268.3	3.5	271.4	268.9	3.7

[1] bST=bST treatment, CON= control (no bST), SEA 1= season 1 (October-March), SEA 2= season 2 (May-September), MSE= mean standard error.

[2] Within 24 h after calving.

[ab] Means with different letters within rows and category differed ($P<0.1$)

Table 7. Least squares means and MSE for overall postpartum BW and BCS, and 150 d MY of Holstein heifers fed diets with different amounts and ratios of protein and energy (CP:ME; g/Mcal) during the prepubertal growth period.

	LL [1]	LH [1]	HL [1]	HH [1]	±MSE
BW (kg)	517.8	506.9	509.7	493.7	6.72
BCS	3.57	3.55	3.62	3.49	0.07
150 d MY (kg/d)	30.0	30.1	28.6	29.9	0.14

[1] LL= low protein-low energy, LH= low protein-high energy, HL= high protein-low energy, HH= high protein-high energy, MSE= mean standard error.

Table 8. Least squares means and MSE for overall postpartum BW and BCS, and 150 d MY of Holstein heifers injected or not injected with bST during the prepubertal growth period and during two seasons.

	bST [1]	CON [1]	±MSE[1]	SEA 1[1]	SEA 2[1]	±MSE[1]
BW (kg)	502.8	511.3	5.31	499.4	514.6	5.26
BCS	3.55	3.57	0.06	3.52	3.59	0.06
150 d MY (kg/d)	28.8	30.5	0.10	29.4	29.9	0.10

[1] bST= bST treatment, CON= control (no bST), SEA 1= season 1 (October-March), SEA 2= season 2 (May-September), MSE= mean standard error.

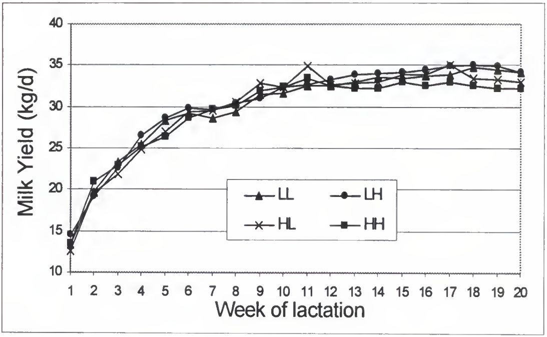

Figure 1. Milk production of heifers fed the four diets. LL= Low protein-Low energy, LH=Low protein-High energy, HL= High protein-Low energy, HH=High protein-High energy.

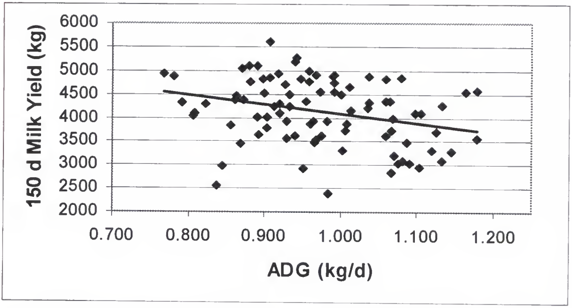

Figure 2. Linear regression of prepubertal average daily gain (ADG; kg/d) and 150 d total MY adjusted for effects of diet, bST and SEA. Y=6042.1 - 1966.5X; R^2=0.0781.

reported by Abeni et al. (2000). They found no significant differences in MY when they fed heifers diets formulated to achieve standard (100% of NRC, 1989 recommendations) or accelerated (115% of NRC, 1989 recommendations) growth rates. These diets contained either 57.3 or 59.3 g CP:ME Mcal and were fed during the prepubertal period (from 150 through 300 kg BW) or either 45.5 or 50 g CP:ME Mcal during the postpubertal period (for 7 mo after reaching 300 kg BW). Their findings contrast those reported by Radcliff et al. (2000). These latter researchers fed heifers two diets with either standard (17.5% CP and 2.3 Mcal/kg) or high CP and energy (19.3% CP and 2.8 Mcal/kg) from 135 kg of BW until pregnancy. Although they increased the percentages and quantity of protein and energy in the diet, the CP:ME ratio decreased from 76.1 to 68.9 g/Mcal. They reported 270-d MY tended to be greater (P=0.08) for standard fed heifers (29.1 kg/d) compared to those fed the high CP and energy diet (25.6).

VandeHaar (1998) later suggested that protein and energy recommendations needed to be reevaluated, relative to effects on growth of mammary gland tissue and MY. This evaluation could be depicted as a ratio between these two factors (g CP:Mcal ME), or the actual amount of MP fed. When MP was calculated and used in place of CP, the MP:ME ratio accounted for 88% of the variation in parenchymal DNA responses. The ratio of these two factors (MP and ME) appeared to be a better predictor of animal performance than either one alone, or the two together. Indeed, earlier studies of Oldham (1984) indicated that the associated effects of protein and energy could affect nutrient use.

The current experiment showed increased ADG (Table 3) of heifers as the CP:ME ratio (g/Mcal) increased (Table 1) except for LL which, although having slightly greater CP:ME ratio, had slightly lower ADG than LH. This discrepancy can be explained, in part, by the calculation of MP which then is used in place of CP for the protein:energy ratio. The ratio of MP:ME (g/Mcal) was less for LL than for LH, which may explain lower ADG for LL. Even though no significant linear effect was found between MP:ME ratios and ADG, results agreed, in part, with results of Bagg et al. (1985), Lammers and Heinrichs (2000) and Gabler and Heinrichs (2003). These studies showed that ADG increased when protein content of the diet was increased. Lammers and Heinrichs (2000) reported maximum feed efficiency for growth at a CP:ME ratio of approximately 60 g of CP/ Mcal ME. Our results indicated that as MP:ME (g/Mcal) ratio in the diet increased, ADG increased and reached a plateau at around 46 g MP:Mcal ME (P=0.0433).

The NRC (1989) recommended diets with CP:ME ratios between 59 and 62 g CP/ME Mcal be fed to heifers up to 6 mo of age and 49 to 51 g CP/ME Mcal be fed from 6 to 12 mo of age. However, Lammers and Heinrichs (2000) reported improved ADG, feed efficiency, and better mammary ductal development (through indirect measurements) for diets with CP:ME ratios of 60.9 (16.6% CP) compared to either 46.3 (11.8% CP) or 54.2 g (13.8% CP) CP/Mcal ME.

When simple correlations between ADG and MY, independent of diet, bST or season effects, were calculated, a significant negative correlation (Pearson correlation coefficient = -0.27898; P=0.0085) was detected; there was a significant decrease in milk yield as ADG increased (Figure 2). Similar results were found when simple correlations between ADG and MY were calculated for each diet treatment (Figure 3). Mean age at calving for these heifers was between 22 to 24 mo of age at mean prepartum BW of 621.0 ± 20.9 kg, which was within current recommendations (Hoffman and Funk, 1992; Hoffman; 1997).

Prepubertal bST treatment had no significant effects on any of the variables measured up to 341 kg BW (Table 4) or after diets had been discontinued when heifers BW reached 341 kg (Tables 6 and 8). Although no effect of bST on ADG was detected, bST injections tended to increase ADG (0.998 vs. 0.960 kg/d) and the heifers remained on treatment an average 7 d less even though they weighed less when they first started on the experiment (114.7 vs. 121.3 kg BW for bST and CON groups, respectively) . No evidence of delayed maturity was found because all heifers calved at similar ages (Tables 5 and 6) independent of diet or bST injections. Similar findings were reported by McShane et al. (1989), Vestergaard et al. (1993), Radcliff et al. (1997) who reported that prepubertal injections of bST increased growth rates through puberty but had no effect on age at puberty. Body weight alone can be misleading as a size descriptor for an animal. An animal can be short and fat and weigh just as much as an animal that is tall and thin. Heifers in the

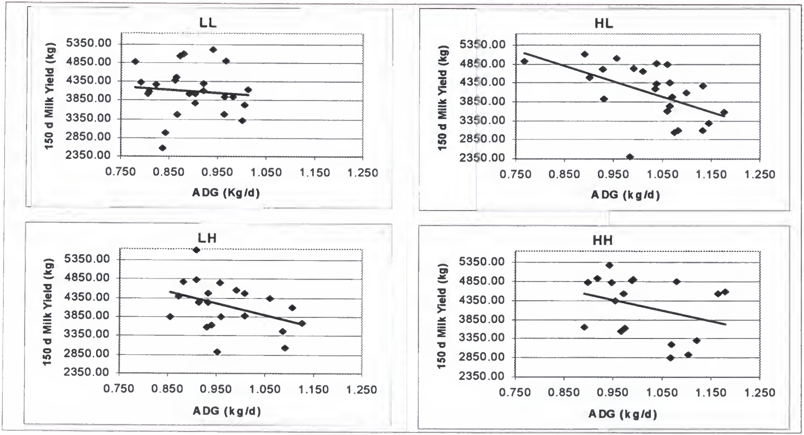

Figure 3. Linear regressions of prepubertal average daily gain (ADG; kg/d) and 150 d total MY of growing heifers fed diets with different protein (14 or 18% of DM) and energy levels (2.75 or 2.86 Mcal/kg ME) during the prepubertal growth period (125.2 ± 3 d through 341 kg BW). LL= low protein-low energy, Y=4813.3 - 795.87X, R^2=0.0075; LH=low protein-high energy, Y=7170 - 3128.6X, R^2=0.1541; HL=high protein-low energy, Y=8243.6 - 4037.8X, R^2=0.2681; HH= high protein-high energy, Y=6980.4 - 2745.6X, R^2=0.098.

current experiment showed no significant differences in final height of bST-treated heifers (123.7 cm) and controls (123.9 cm), which eliminated possible misleading conclusions related to size of the animal, as mentioned above.

The rate of mammogenesis during the prepubertal phase is directly influenced by circulating concentrations of ST (Sejrsen et al., 1986). Radcliff et al. (1997) reported that bST treatment (25 μg/kg BW) increased mammary parenchymal DNA, RNA and the ratio of RNA to DNA. Increases in mammary parenchymal DNA, RNA and the ratio of RNA to DNA have been found to result in greater MY (Capuco et al., 2001). We speculated that MY could be increased by administrating bST during the prepubertal period, possibly due to increased quality of parenchymal tissue. In the current study, prepubertal bST treatments did not affect 150-d MY (P=0.1788); means for injected and not-injected heifers were 28.8 kg/d and 30.5 kg/d, respectively (table 8). Heifers in this study were not sacrificed so conclusions related to quality and quantity of parenchymal tissue could not be made. Possible explanations for the lack of significant response to bST treatment during the prepubertal period on milk yield could be: first, no effect on mammary parenchymal tissue growth occurred; second, bST might have increased mammary parenchymal tissue growth but without subsequent increases in MY; third, the dose used was not adequate; fourth, although undetected on this study, diet might have influenced the results.

Overall, results of this study suggest that diets fed to heifers during the prepubertal period containing CP:ME ratios between 50 and 70 g/Mcal, or ratios of MP:ME between 41and 46.7 g/Mcal may alter growth rates but will not affect MY. Additionally, bST treatments during this period may be useful for increasing growth rates but it did not provoke increased milk production during first lactation.

CHAPTER 3

MILK PRODUCTION AND PHYSIOLOGICAL RESPONSES OF MULTIPAROUS HOLSTEIN COWS FED DIETS SUPPLEMENTED WITH GLUCOGENIC COMPOUNDS DURING THE TRANSITION PERIOD

Introduction

The transition period for dairy cows has been defined as the time period between 3 wk prepartum and 3 wk postpartum. During this 6 wk period, cows undergo metabolic changes that may have a profound impact on their overall performance, not only during the transition period but also during the following lactation. Due to metabolic and gastrointestinal tract fill constraints, intake of energy by cows during the transition period is restricted and this normally results in a period of negative energy balance before they calve (Bertics et al., 1992). Dry matter intake (DMI) can decline as much as 30%, or even more before calving, and increases slowly during early lactation. The decrease in DMI may result in a negative energy balance because of increased energy demands to support the requirements of the fetus and uterus during the prepartum period, and the increase in milk synthesis postpartum. In order to minimize and overcome this energy deficit, cows must rely on their stored lipids as an important source of energy. This is especially true during early lactation. Metabolic disorders may result if large quantities of lipids are mobilized (Grummer, 1995). Without taking economic losses that are due to suppressed production and reproduction failure into consideration, health costs have been estimated to be five times greater during early lactation than during mid- and late lactation (Young et al., 1985).

Supplying glucogenic precursors during the transition period may be one management strategy to increase glucose supply to reduce extent of increases in non-esterified fatty acids (NEFA) and β -hydroxy butyrate (β-HBA), and to prevent associated metabolic disorders (Burhans and Bell, 1998). For this reason, objectives of this study were to evaluate DMI, physiological and production responses when supplemental glucogenic precursors were included in the diet from 3 wk prepartum through 4 wk postpartum, and to evaluate any benefits on health throughout this period and the subsequent lactation.

Literature Review

Feed Intake During Transition and Related Health and Metabolic Disorders

To date many equations have been developed to predict DMI. Most models proposed to predict voluntary DMI (VDMI) of lactating cows were developed empirically by applying multiple regression techniques (Ingvartsen, 1994; Forbes, 1995). Several studies (Fuentes-Pila et al., 1996; Roselar et al., 1997) have shown inaccuracies of these equations when they were evaluated by using independent data sets. Current NRC (2001) DMI prediction equations are based only on animal factors that were evaluated with treatment means from experiments published in the *Journal of Dairy Science* between 1988 and

1998. Fuentes-Pila and co-workers (2003) indicated that the NRC equations should include additional factors that consider both dietary and animal factors as variables to adjust intake results. Their proposed equation better predicted DMI than the older versions of the DMI prediction equations of the NRC (1989). The newer version adjusted results slightly better when it was tested using data sets published in the *Journal of Dairy Science* during 1985 to 1989. This suggested that there were diverse factors that influenced DMI and that formulating a precise and accurate prediction equation would require additional research.

Factors affecting and regulating DMI of dairy cows are numerous and complex and span cellular to macro-environmental levels (Forbes, 1996; Allen, 2000). Some factors may be controlled by humans and among these are animal factors (i.e., age, BCS, breed, physiological stage, and MY), dietary factors (i.e., ingredient and nutrient composition of diets and physical and agronomic characteristics of feeds), managerial factors (i.e., production, feeding and housing systems), and climate factors (i.e. temperature, humidity and wind). Factors affecting DMI of dairy cattle have received much attention for many decades, especially for lactating cows. The traditional motivation for studying factors affecting DMI has been and still is the possibility of increasing intake and hence production efficiency and profitability (Ingvartsen and Anderson, 2000).

The dry period once was considered by some researchers (Van Saun, 1991; Noceck, 1995) as a non-profitable resting period. However, epidemiological surveys ascertained that dry period nutrition had carryover effects on milk yield and reproductive performance during early lactation and health status during the periparturient period (Curtis et al., 1985; Erb and Grohn, 1988; Correa et al., 1990). Zamet and co-workers (1979) indicated that most health problems of both metabolic and infectious nature in highly selected dairy cows occurred during early lactation and were related to relatively low DMI just prior to parturition. Pregnancy in dairy cows reduced DMI by 1.53%/ wk from wk 26 of pregnancy through 3 wk before calving (Ingvartsen et al., 1992). During the final 3 wk of gestation DMI tended to decrease about 32%, and 89% of this decline occurred during the final week of gestation (Hayirli et al., 2002). Figure 4 depicts expected DMI decline of dairy heifers and cows during the transition period (adapted from Ingvartsen and Anderson, 2000). Several mechanisms likely are involved in the decline of VDMI during the prepartum period. Some researchers have suggested that the decline is caused by the physical compression of the rumen by the growing uterus and/or that there is hormonal control. Research has described the involvement of these two factors in VDMI. These and other factors have been reviewed in detail by Hayirli et al. (2002) and Ingvartsen and Anderson (2002).

In cows fed diets of constant composition, the milk yield typically peaks between 5 and 7 wk postpartum, whereas maximum DMI is reached between 8 and 22 wk after calving. The 20-40% gradual decline in VDMI during the final 3 wk of gestation may initiate a negative energy balance which may compromise the ability of the cow to adapt to physiological changes at this time

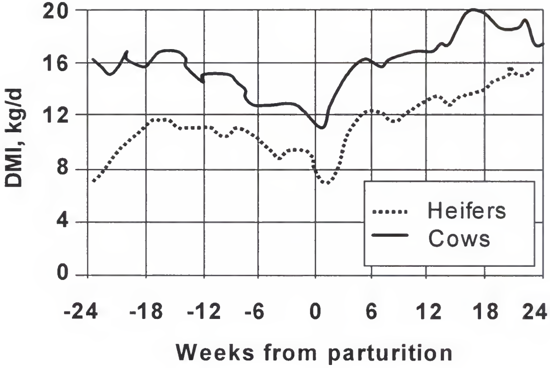

Figure 4. The pattern of transient dip in voluntary dry matter intake (DMI) around calving in heifers and cows (adapted from Ingvartsen and Anderson, 2000)

(Grummer, 1995). Failure of the cow to adapt to physiological changes results in manifestations of metabolic disorders and/or health problems such as displaced abomasum (DA), fatty liver, milk fever (hypocalcemia), retained placenta (RP) and ketosis. Occurrence of these health problems, which may result in a decrease in MY, BW and BCS, also may retard recurrence of ovarian cycle activity, hence reducing overall profitability. About one-half of multiparous dairy cows experience moderate to severe fatty liver after calving (Grummer, 1995). Fatty liver also has been associated with increased incidence of mastitis and DA (Herdt, 1988), RP (Bruss, 1993), poor reproductive performance (Gerlof et al., 1986), and immune suppression (Reid et al., 1984). Cows with fatty liver are more prone to develop ketosis (Bruss, 1993) and suffer production losses (Grummer, 1993). Wallace et al. (1996) reported that cows in their study that had any of the health disorders around calving produced 7.2 kg/d less milk during first 20 d postpartum than did healthy cows.

Goff and Horst (1997) indicated that most of the metabolic disorders (milk fever, ketosis, RP, and DA) occurred during the first couple of weeks after parturition and were seldom fatal, but that those metabolic diseases that were not clinically apparent during the first 2 wk of lactation, such as laminitis, could be traced back to insults that occurred during early lactation.

Chronic deficiencies of energy, protein, minerals, or vitamins have been associated with increased disease susceptibility as a result of depressed immune function. The great metabolic stress imposed on the cow at parturition and the onset of lactation caused relative acute deficiencies of nutritional factors that were necessary for maintenance of the immune system. These deficiencies could last from one day to several weeks (Goff and Horst, 1997). Severe energy deficiencies during early lactation also could cause ketone bodies to accumulate in blood that further impaired lymphocyte function (Franklin et al., 1991). Immunosuppression during the periparturient period led to increased susceptibility to mastitis. At parturition, plasma concentrations of vitamin A (retinol) and vitamin E are reduced by 38 and 47%, respectively (Goff and Stabel, 1990). Supplementation with vitamins A and E improved immune responses of the periparturient cows (Daniel et al., 1991; Stabel et al., 1992; Hogan et al., 1993). Also, supplementation with vitamin A and E often was associated with decrease in the incidence of mastitis in dairy cows (Smith et al., 1984; Hogan et al., 1993).

High energy demands during the transition period together with depressed DMI and low glucose availability leads to rapid mobilization of fatty acids (FA) from adipose in the form of NEFA (Figures 5, 6). Although NEFA can be metabolized by many tissues, a large portion is extracted from blood by the liver which receives much of the total blood flow and has an efficient NEFA extraction mechanism. Once NEFA are in the hepatocytes they may follow any of three possible metabolic pathways. The first of these is complete oxidation for energy production, however, the hepatic requirements for energy are such that only a small amount of the total FA supply during adipose mobilization needs to be used for complete oxidation. The second is esterification of FA for

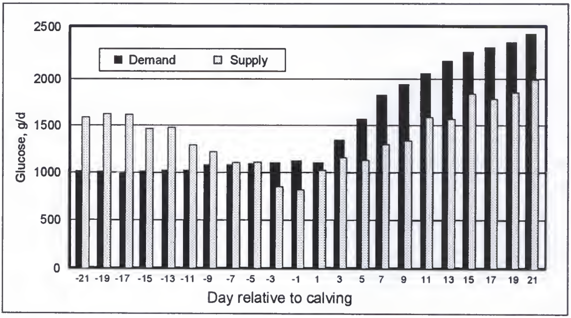

Figure 5. Predicted whole body glucose demand and supply during the transition period (adapted from Overton, 2001).

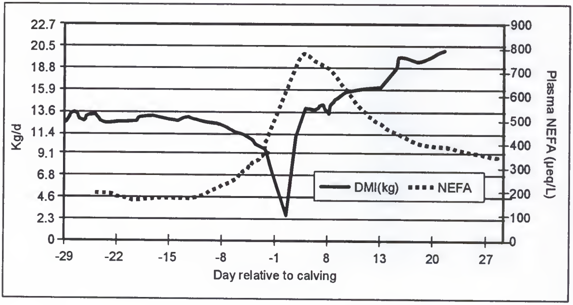

Figure 6. Relationship between dry matter intake (DMI) and NEFA concentrations in plasma during the transition period (adapted from Overton, 2001).

synthesis of triglycerides. The third pathway is partial oxidation to produce ketone bodies. Formation of ketone bodies occurs within the hepatic mitochondria and the rate of ketone body synthesis is controlled by the regulated transport of FA across the mitochondrial membrane. Carnitine palmitoyltranferase 1 (CPT-1) is the enzyme that determines the rate at which FA are transported into the mitochondria. Together, the activity of this enzyme and the availability of FA are the primary determinants of the rate of ketone body formation. Although ketone bodies are produced in the liver, they can not be used there for energy production. Therefore, all ketone bodies are transported to peripheral tissues for utilization (Figure 7).

Ketosis is a metabolic disorder that occurs when the concentration of ketone bodies in the blood becomes abnormally high. During the period of high energy demand, concentrations of NEFA in the circulation increase and thus more become available to be used by the liver hepatocytes. As mentioned previously, the amount of NEFA oxidized to carbon dioxide to provide energy for liver function is relatively small. Most of the unutilized NEFA can go in one of two directions. The CPT-1 enzyme activity is highly regulated and when it reaches its maximum a greater proportion of the NEFA are directed towards esterification to form triglycerides (TG) or phospholipids.

Ruminants export TG from the liver in the form of very low density lipoproteins (VLDL), but only in limited amounts. If NEFA availability and uptake by the liver continues to increase, then the synthesis of TG in the liver also will increase and this leads to its accumulation. The resulting syndrome is

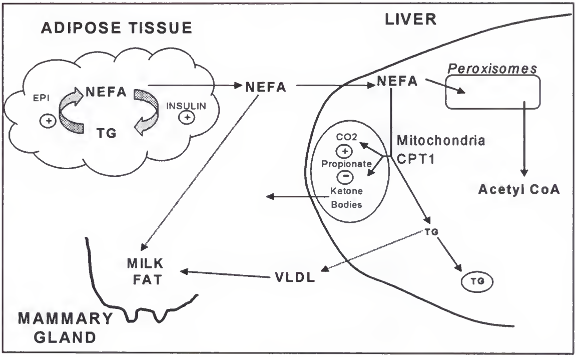

Figure 7. Schematic representation of relationships of lipid metabolism in adipose tissue, liver, and mammary gland. Plus signs (+) indicate stimulatory effects, minus signs (-) indicate inhibitory effects. Dashed lines indicate processes that occur at low rates or only during certain physiological states. Abbreviations: EPI= epinephrine, TG= triglyceride, VLDL= very low density lipoproteins, CPT-1 = carnitine palmitoyltransferase 1 (adapted from Drackley, 1999).

hepatic lipidosis or fatty liver (FL). Increased lipid accumulation and decreased glycogen accumulation in the liver have been associated with increased susceptibility to the development of ketosis (Drackley et al., 1992). The exact nature of the impact of lipid infiltration on normal hepatic functions is not clear and contradictory conclusions have been reported. However, evidence suggests that fat infiltration *per se* does not affect gluconeogenesis in bovine hepatocytes (Strang et al., 1998). However, TG accumulation in hepatocytes decreases the capacity for urea synthesis (Strang et al., 1998) and ammonia decreases the ability of hepatocytes to synthesize glucose from propionate (Overton et al., 1999). Therefore, TG accumulation may indirectly inhibit glucose synthesis in cows. The inability of the liver to detoxify ammonia by conversion to urea and glutamine have been related to decreased reproductive performance (Drackley, 2003).

Other studies have linked fatty liver (FL) to increased incidence of metabolic and infectious diseases (Reid and Roberts, 1982; Rukkwamsuk et al., 1999; Heuer, 2000). It has been hypothesized that FL may increase the incidence of milk fever by altering liver metabolism of vitamin D (Reid, 1982). Cows with FL may have decreased capacity to synthesize protein in hepatic cells, as indicated by decreased amounts of rough endoplasmatic reticulum in liver cells and decreased concentrations of albumin in blood (Reid and Collins, 1980). It has been reported that cows with FL retained viable pathogenic bacteria in the mammary gland for a longer period than control cows after experimental infection. The possible mechanisms by which FL might decrease immune function are unclear. As mentioned previously, FL impairs glutamine synthesis which is an essential amino acid for lymphocytes (Newsholme et al., 1985). Another important relationship with the immune system is the liver acting as a barrier to prevent intestinally derived endotoxins from entering peripheral circulation. For FL in laboratory animals, this barrier function was compromised, which led to endotoxemia (Nolan, 1989).

Various metabolic disorders and the negative energy balance were reported to be associated with the development of DA (Muylle et al., 1990). For example, Heuer (2000) reported that 53% of the Left DA (LDA) cases were associated with the effects of decreased DMI and negative energy balance. Left DA is a disorder that occurs mainly in high producing postpartum dairy cows. The early postpartum period is considered to be the major risk period, because hypocalcemia, metritis, negative energy balance, as well as nutritional factors, play a central role in the pathogenesis of LDA. Three factors are thought to be responsible for allowing the abomasum to move to the left side of the cow. First, the rumen must fail to take up the void left by the retracting uterus. Second, the omentum attached to the abomasum must have been stretched to permit movement of the abomasum to the left side, and third, there is abomasal atony (Goff and Horst, 1997). Decreased DMI reduces fill in the rumen which increases the risk of LDA. Decline in plasma calcium concentration linearly decreases abomasal contractility, which is suspected to lead to atony and distension of the abomasum. Additionally, lower DMI and/or diets high in grains can promote the appearance of VFA in the abomasum because there is reduced depth of the rumen mat; VFA within the abomasum also reduce abomasal contractility.

Improved Energy Balance and Reduction of Metabolic and Health Problems During the Transition Period.

Cows have a great demand for glucose, especially postpartum (1,100 g/d for pregnant cows; 2,500 g/d postpartum), but the net absorption of glucose from the gastrointestinal tract is very low in ruminants. Instead, ruminants rely on some non-glucose precursors from which to synthesize glucose. The two organs with glucogenic capacity are liver and kidney. Most carbohydrate digestion in ruminants occurs in the fore-stomach through fermentation. The result is that essentially no digestible carbohydrates reach the intestines for digestion and absorption as glucose. Ruminants are in constant state of potential glucose deficiency. This condition forced ruminants to develop efficient systems for production and conservation of glucose.

Quantitatively, the most important glucogenic precursor is the volatile fatty acid (VFA) propionate. Propionate contributes to glucose synthesis after it is absorbed by entering the Krebs cycle at the level of succinate. Bell (1995) suggested that cows can mobilize body protein (i.e., from muscle) and utilize some glucogenic amino acids to synthesize a portion of the additional glucose needed by cows during early lactation; dietary amino acids also contribute to the total pool of amino acids used by the liver. Overton et al. (1998) indicated that the capacity of the liver to synthesize glucose from amino acids increases substantially during the first 21 d of lactation. Use of amino acids to synthesize glucose is not energetically efficient, but may explain why feeding diets containing more than the NRC (1989) specified requirements of 12% CP in the diet of early lactation cows has proven to be effective in practice. Some amino acids that may be used for gluconeogenesis are valine, threonine, methionine, isoleucine, glutamate, histidine, proline, arginine, alanine, glycine, serine, tyrosine, and phenylalanine. The two VFA, acetate and butyrate, also enter the Krebs cycle as acetyl CoA but this does not lead to production of glucose. From the energy sources produced by fermentation that are available in ruminants (VFA; acetate, propionate, and butyrate) only propionate can support glucose production. Butyrate goes mainly to ketone body formation and acetate to fatty acid synthesis or they are used to provide energy.

Most glucose in the rumen is used by rumen microorganisms, whereas almost all propionate absorbed by reticulo-rumen mucosal cells is delivered to liver through the hepatic portal vein (HPV). Other carbon sources for glucose synthesis include lactate and glycerol. Lactate utilization for gluconeogenesis primarily represents recycling of carbon because most circulating lactate is formed during catabolism of glucose by peripheral tissues or by partial catabolism of propionate by visceral epithelial tissues. The skeletal muscle lacks the enzyme glucose 6-phosphatase, therefore, lactate is produced in muscles instead of glucose and so the lactate returns to the circulation and is recovered by the liver where it then can be converted back to glucose.

Overton (2003) indicated that "most of the determination of how dairy cows will approach peak milk yield is based upon what happens during the last third of the previous lactation". This includes the prepartum portion of the transition period (the last 3 wk before calving). Similarly, metabolic disorders such as milk fever, fatty liver and ketosis have their roots during this transition period and results in substantial economic losses. Furthermore, cows that get off to excellent and healthier start following calving are more likely to be more successful in terms of reproductive performance. Therefore, the potential benefits of optimizing all aspects of cow management during this time period are substantial.

The start-point of most metabolic disorders, which result in decreased milk production and overall profitability, is the decrease in DMI while energy demands increase. This results in FA mobilization and excess TG deposition in liver. Prepartum DMI is related to the extent of TG deposition in the liver by the time of calving (Bertics et al., 1992). This association likely reflects negative energy balance and FA mobilization from adipose tissue. One of the primary concerns for dairymen is to maintain DMI of transition cows at adequate levels which ensures that all the nutrients required by the cow are available and supplied in the diet fed. Given the large degree of cow variation in DMI depression prior to calving, factors that influence prepartum DMI need to be identified and quantified. Even after managing all variables involved in regulating DMI, energy demands still may be much greater than can be provided in the diet and/or consumed by the cow.

Over a period of years researchers tested various approaches to overcome, or reduce as much as possible, the negative energy balance that cows suffer during transition. Feeding prepartum diets that are more energy dense than formerly were recommended by NRC (1989) has resulted in reduced concentrations of plasma NEFA and lipid infiltration of the liver (VandeHaar et al., 1999). These two measures are good indicators of energy status. A number of glucogenic compounds also have been tested to evaluate their ability to reduce energy deficit, increase DMI and/or improve availability of glucose, and to reduce lipid mobilization and storage in the liver of transition cows.

Propionate is the principal VFA that results from fermentation in the reticulo-rumen and its production depends upon DMI. The major site of propionate metabolism is the liver, which takes up to 80% of the blood propionate load in one pass (Van Soest, 1994) and up to 95% of the total during multiple passes (Bergman, 1990). Studies with labeled propionate showed that about 50% is converted to glucose with a portion appearing as lactate (Annison and Armstrong, 1970). However, Steinhour and Bauman (1986) estimated that this value was nearly 90%. Their explanation for this higher estimate was that there was a considerable cross over of carbons that underestimated the final value. Importantly, propionate supplementation was found to increase blood glucose concentrations (Baird et al., 1980) and lower β-HBA (Goff et al., 1996)

Feeding or injecting Ca or Na salts of propionate markedly decreases the levels of blood ketone bodies. Grum et al. (1996) indicated that propionate was a potent inhibitor of lipid oxidation and it decreased liver oxidation of

NEFA (Armentano et al., 1991). A Florida research group (Melendez et al., 2002) treated Holstein cows with single doses of anionic salts (calcium chloride, calcium propionate + PPG, or calcium borogluconate) at calving. They reported that single doses of these salts had no effects on Ca, P, Mg, NEFA, β-HBA, or glucose concentrations. Similar results were reported by Johnson et al. (1998) when cows were treated with 200 ml of molasses, 349 g of calcium propionate + 200 ml of molasses, 60 g of MgO in molasses, or two doses (at calving and 24 h later) of 349 g of calcium propionate + 200 ml of molasses. Calcium propionate increased blood glucose by 11% but for less than 3 h duration. They concluded that drenching calcium propionate for prevention of subclinical hypocalcemia and ketosis in early lactation was of little value. On the other hand, Schultz (1958) had reported increased MY and blood glucose concentrations at 21 and 35 DIM when cows were fed 113 g of sodium propionate per day from calving through 42 DIM. Additionally, blood ketone body concentrations were less at 7, 21, 35, and 49 DIM. Goff et al. (1996) reported a tendency for calcium propionate to lower β-HBA at 2 and 10 DIM. Taken together these data suggest that calcium propionate may have beneficial effects on transition cows if supplemented for long periods of time and not just supplemented as single doses.

Feeding supplemental fat to cows during early postpartum period has been considered to be beneficial because diet energy density was increased and body lipid mobilization was reduced. It has been suggested that if fat is incorporated into transition diets that it should be introduced gradually into the diet during the last 2 or 3 wk before parturition to accustom cows to the fat and prevent any off-feed problems. Some studies (Drackley and Schingoethe, 1986; Jerred et al.,1990) have shown that the response to dietary fat is delayed until to 5 to 10 wk postpartum when fed during early lactation. Drackley (2003) suggested that delayed response could be due to the extensive mobilization of FA as NEFA from adipose tissue during the period of negative energy balance that cows undergo, and this could exert a negative feedback on feed intake (Grummer and Carroll, 1991). The mechanisms by which dietary fat would minimize the risk of fatty liver, ketosis or both and improve energy balance would include reduced FA mobilization from adipose tissue. This would alleviate a shortage of FA precursor for mammary TG synthesis, and this would spare mammary glucose oxidation because of reduced requirement of $NADPH^+$ for mammary fatty acid synthesis. Kronfeld (1982) also hypothesized that supplemental fat alleviated ketosis because of increased quantities of FA precursors for milk fat synthesis and reduced FA mobilization from adipose tissue. This explanation assumed that dietary FA were incorporated into lipoproteins synthesized in the intestine and, in contrast to NEFA, were metabolized predominantly by extrahepatic tissues. In contrast to Drackleys' hypothesis, Grummer (1993) suggested that the increase in NEFA when cows were supplemented with fat resulted from an increase in NEFA release from adipose tissue or from incomplete tissue uptake of NEFA following hydrolysis of lipoprotein TG by lipoprotein lipase.

Feeding rumen degradable fat apparently interferes with rumen fermentation with subsequent depression of DMI when fed in excess of about 5% of the diet DM. Addition of fat to diets fed to lactating cows increased energy intake in support of higher milk yields, provided that it did not reduce DMI. The recommended maximum fat concentration in diets fed to lactating cows was 6 to 7% of dietary DM (NRC, 1989), but this limit was established for cows yielding 20 to 30 kg milk/d and was based on inhibition of ruminal metabolism and decreased efficiency of milk synthesis by higher fat percentage. However, when dietary fat was increased from 6.1 to 8.6% of diets fed to lactating cows that yielded aproximately 40 kg/d of milk it did not enhance milk yield, but it did reduce DMI (Elliott et al., 1993). Dietary FA at 9% of diet DM may result in reduced FA digestibility (Khorasani et al., 1992) because of limited biliary and pancreatic secretions into the small intestine, although these were not determined in the study of Elliott et al. (1993). The merit of feeding rumen inert fat is that it may not interfere with rumen fermentation and that when the fat arrives at the small intestine it enters the lymphatic system as chylomicrons. Chylomicrons do not add to hepatic lipid accumulation because the liver lacks lipoprotein lipase (Emery et al., 1992). On the other hand, the mammary gland has abundant lipoprotein lipase (Havel, 1987).

Wu and Huber (1994) studied the effects of increasing fat from 3 to 7% by using a combination of whole cottonseeds and ruminally resistant fat (prilled tallow fatty acids) or safflower oil, which is highly unsaturated and not resistant to ruminal hydrolysis. They reported that milk yield increased 2.1 kg/d on average by addition of 2.2% prilled tallow fatty acids or safflower oil (7% fatty acids) to the diet that contained 12% whole cottonseeds. However, when fatty acids were increased to 9.1% by supplementing additional prilled tallow fatty acids, milk yield, DMI, and fatty acid digestibility decreased. They concluded that fat included in diet at 9% of dietary DM appeared to be excessive for cows producing 30 to 40 kg milk /d.

Although ruminants absorb considerable amounts of VFA, mainly acetate, propionate, and butyrate from the forestomach, they absorb little glucose from the intestinal tract (Herdt, 1988). Thus, circulating glucose in ruminants is derived from gluconeogenesis that occurs primarily in the liver (Young, 1977) with extent of gluconeogenesis dependent largely on the availability of glucogenic precursors. In fed ruminants, propionate is the major precursor for gluconeogenesis, but glycerol and glucogenic amino acids are the predominant source in ruminants deprived of feed.

Three key enzymes important in hepatic gluconeogenesis are phosphoenolpyruvate carboxykinase (PEPCK), fructose 1-6 biphosphatase (FBPase), and glucose 6-phosphatase (G6-Pase). Rukkwamsuk et al. (1999) studied effects of overfeeding pregnant cows on activity of enzymes in liver. They overfed 7 pregnant cows during the dry period and compared them to 6 control cows by analyses of blood and liver samples taken at -1, 0.5, 1, 2, and 3 wk from calving, for activities of PEPCK, FBPase, and G6-Pase. Their results showed that overfed cows were less well adapted for gluconeogenesis before parturition and PEPCK activity was decreased. They concluded that overfed cows that had livers infiltrated with fat had less than optimal rates of gluconeogenesis and that this resulted in a more prolonged lipolysis, particularly during first several weeks after parturition.

It is a common management practice among dairymen to feed diets with increased concentrations of energy, protein, and certain minerals in close-up dry diets (CUD diets) during the last several weeks of gestation. The goal of feeding CUD diets is to meet their current needs at a time when abrupt physiological changes occur. The common term for this practice is "Steaming up". With this practice greater concentrations of degradable starch are provided which results in improved postpartum performance, mainly due to changes in rumen microflora, improved nutrient supply to ruminal bacteria, and improved nutrient utilization. Nevertheless, introduction of high concentrate diets to cows abruptly, or in excess, also may have negative effects (e.g., acidosis and reduced DMI, among others).

Other approaches for improving energy balance or increasing gluconeogenesis especially during the transition period are inclusion of glucogenic supplements in the diet of transition cows. Some that have been used and evaluated critically are glycerol, calcium propionate, sodium propionate, and propylene glycol. Glycerol may be an important glucogenic precursor as the cow adapts to lactation. Glycerol supply and its potential contribution to gluconeogenesis is entirely dependent on the amount of adipose tissue TG mobilized at any given time during the periparturient period.

Fisher et al. (1971) postulated that glycerol was an appetite stimulant. Importantly, of the four major glucose precursors (propionate, glycerol, lactate and amino acids) commonly evaluated and considered as diet supplements only glycerol can be metabolized to glucose without the need of oxaloacetate. It enters the glucogenic pathways at the level of dihydroxyacetone phosphate and 3-phosphoglyceraldehyde. Goff and Horst (2001) fed dry cows either 1, 2 or 3 L of glycerol to study its effect on blood glucose. Blood samples were taken hourly for 8 hr after feeding and also at 24 hr. At 0.5 h after treatment mean blood glucose increased 16, 20, and 25%, respectively over pretreatment values. Plasma glucose remained elevated for the next 8 h and returned to baseline values at 24 hr. They suggested that glycerol offered another way to treat ketosis which may have less toxic effects than feeding or drenching with propylene glycol (PPG).

Propylene glycol (1,2-propanediol) is the glucogenic precursor most studied in recent years. Propylene glycol supplementation to cows around calving had positive effects. Overall, it increased blood glucose (Waldo and Schultz, 1960; Studer et al., 1993) and insulin concentrations prepartum, and decreased plasma NEFA and β-HBA (Studer et al., 1993). Grummer et al. (1994) drenched PPG orally once daily for 5 d in feed-restricted springing heifers and reported increased plasma glucose and serum insulin and decreased NEFA and β-HBA concentrations. Reviews from the 1960's on the metabolism of PPG indicated that it was oxidized to lactaldehyde and then to lactic acid by several enzymes with low specificity (Huff, 1961; Miller and

Bazzano, 1965). Emery et al. (1967) indicated that PPG was predominantly absorbed from the rumen without alteration, although some conversion to propionic acid in the rumen also was detected. Propylene glycol was metabolized in the liver by alcohol dehydrogenase to lactic acid and then to pyruvic acid. Both of these metabolites are normal constituents of the citric cycle and that are further metabolized to carbon dioxide and water.

More recently Grummer et al. (1994) found a shift in the molar percentages of ruminal VFA when propylene glycol was fed. The most significant shifts were linear decreases in acetate and linear increases in propionate that were due to increasing PPG dose. The shifts in ruminal VFA agreed with data of Waldo and Schultz (1960) and suggested some conversion of PPG to propionate in the rumen.

Oral drenching of cows with PPG is difficult and may not be practical in large dairy operations when used as a prophylactic treatment to reduce occurrence of ketosis around time of calving. However, there has been interest in adding PPG directly in the diet fed which would be an easier management strategy to implement. Christensen et al. (1997) supplemented an average of 324 ml/d of PPG as either an oral drench, mixed with concentrate and fed separately from forage, or blended as part of the total mixed ration (TMR). They found that PPG reduced the molar percentage of acetate and the ratio of acetate to propionate in rumen fluid. Overall, the three methods of delivery resulted in higher concentrations of plasma glucose and insulin, and lower concentrations of NEFA. Feeding PPG mixed with the concentrate was nearly as effective as the oral drench, but PPG in TMR was not as effective as the other two methods of delivery. It is likely that greater quantities of PPG would need to be mixed in the TMR to obtain a beneficial effect, but this has not been quantified.

Monensin is an ionophere antibiotic that alters rumen VFA production; the molar proportion of propionate in the rumen VFA is increased leading to an increased rate of gluconeogenesis in the liver (Bragg, 1997). Additionally, propionate has a benefit on ruminal mucosa papillae development leading to enhanced glucose precursor flow into the blood. Feeding Monensin increased glucose flux without an apparent increase in plasma glucose concentrations. Arielli et al. (2001) suggested that this might be explained by a stimulation of insulin release, that resulted in reduced plasma glucose concentrations due to increased partitioning of glucose to higher priority energy consuming organs such as mammary gland and uterus. Monensin supplementation increased blood glucose and reduced NEFA and β-HBA concentrations when fed to transition cows (Duffield et al., 1998a). Higher blood urea level prepartum (Stephenson et al., 1997) and postcalving (Duffield et al., 1998a; Green et al., 1999) also were found in Monensin supplemented cows. The effects on urea were thought to result from protein-sparing.

Duffield et al. (2003) reported that Monensin supplement administered precalving significantly improved indicators of energy balance. They suggested that Monensin use for prevention of metabolic disorders in lactating dairy cows is best when it was introduced at the time cows entered the close-up group rather than at calving.

Another two glucogenic supplements currently commercially available for use by dairy producers are Metaxerol™ and NutroCal®. Research to evaluate effects of these two supplements is limited and often under farm field trial conditions and not using rigorous scientific protocols. However, each of these two products contain known glucogenic precursors that have been formulated to be fed to cows.

Metaxerol™ (Pestell; New Hamburg, Ontario, Canada) is a pelleted supplement designed to enhance the energy balance of the dairy cow during the critical period around parturition and early lactation. Some of the ingredients are: calcium salts of long chain fatty acids, forage and roughage products, hydrogenated tallow, animal and vegetable fat, whey dry solubles, sodium propionate, PPG, calcium propionate and niacin supplements. The composition of Metaxerol™'capitalizes on the synergy between certain sources of rumen protected fat, protein and glucose precursors. The principal reason for this suggestion is that fat fed in conjunction with glucose precursors seems to have a synergistic effect because responses of cows to glucose precursors or to fat alone was nearly inconsequential, whereas they were greater when the two were fed together. The manufacturer indicates that when fed to cows at the recommended amount (0.454 kg/d/cow from 2 wk prior to calving through 3 wk postcalving), it helps to balance carbohydrates, provides propionic acid that helps to maintain normal production of glucose in the liver, and thus allows the included fat to be used for energy without excessive ketone production. In addition, the rumen inert fat portion may reduce the body fat mobilized for energy. It also has been suggested that Metaxerol™ in diet would increase DMI and improve energy status.

Sorensen et al. (2003) tested the synergism of glucogenic precursors and fat provided in Metaxerol™ as dietary supplements for transition cows. Supplemented cows received 0.454 kg/d/cow of Metaxerol™, whereas control cows received 0.454 kg/d/cow of a 50:50 mixture of calcium salts of fatty acids and ground barley. Dry matter intake was greater in Metaxerol™ supplemented cows during the prepartum period (-14 d to calving) and blood glucose concentrations were greater during both the pre- and postpartum periods. Total liver lipids tended to be lower (8.1 vs 9.6% wet wt, P=0.17) in the Metaxerol™ supplemented cows. They suggested that the depression of DMI and incidence of ketosis during the periparturient period could be prevented or reduced by supplementing cows with Metaxerol™ during time period recommended starting 2 wk prior to calving through 3 wk postpartum.

NutroCal™ (Kemin Americas; Des Moins, Iowa) is a dry supplement of essential mineral and glucogenic sources. Ingredients include calcium propionate, water, zinc propionate, and copper carbonate, and that it supplied about 0.582 Mcal of ME per kg. Recommendations are that it be fed at a rate of 114 g/d/cow from 3 wk prior to calving through 4 to 5 wk postpartum. The recommended feeding level (114g/d/cow) supplies 88.5 g of propionate, 24.1 g of calcium, 0.35 g of zinc, and 0.12 g of copper and ~0.145 Mcal of ME.

As for Metaxerol™, most research conducted with NutroCal™ has been published by the manufacturer. Spears et al. (2001) studied the absorption and retention of calcium from NutroCal™ in growing steers in a 2x2 factorial design. NutroCal™ was compared to calcium carbonate, and both were fed at two dietary levels, 0.25 and 0.75%. NutroCal™ increased ruminal soluble calcium concentration but no differences were detected in plasma Ca due to Ca source. Supplementing NutroCal™ for 3 wk prior to calving through 4 to 5 wk after calving reduced the incidence of ketosis from 15 to 8%, milk fever from 16 to 5%, and DA from 2 to 0%. These results agreed with report of Goff et al. (2003) where NutroCal™ was supplemented at rates of 0, 0.68, 1.02, and 1.36 kg in 9 L of water administrated using esophageal pump at calving. Drenching cows with NutroCal™ significantly increased blood calcium concentration above 8.0 mg/dL within 2 h and it was maintained at this level through 24 h.

As mentioned previously, limited research has been conducted with these two commercially available glucogenic supplements. To our knowledge, up to this date, no research has been conducted to compare the most commonly available glucogenic supplements available for transition cows under similar experimental conditions. For these reasons objectives of this experiment were to evaluate three glucogenic supplements using multiparous Holstein cows during the transition period, to determine whether including these supplements in daily ration fed would affect their transition DMI, blood metabolites, and infiltration of fat in the liver and their postpartum milk production and reproduction.

Materials and Methods

Experimental Design and Treatments

One-hundred twenty four multiparous Holstein cows completed experiment and were used to evaluate effects of including glucogenic supplements in the TMR fed to transition cows. Cows were assigned randomly to one of four diets 30 d prior to expected calving, but feeding the supplements did not begin until 3 wk prior to expected calving. Treatments were: **CON**= control (no supplement), n=29; **NUT**= NutroCAL™ (Kemin Americas, Des Moins, Iowa), 0.114 kg/d, n=33; **MET**= Metaxerol™ (Pestell America, New Hamburg, Ontario, Canada), 0.454 kg/d, n=31; and **PPG**= propylene glycol, 0.300 mL/d, n=31. In addition to treatment groups, cows were divided into two season groups (SEA 1 and SEA 2) according to the month when they calved (SEA 1=October-March; SEA 2=May-September).

Feeding and Management of Cows

Cows were transferred from the far-off dry group to a free-stall barn equipped with Calan Gates (American Calan, NH) ≅ 30 d before expected calving (≅ one wk before they were offered the supplements) to be trained to the Calan Gate system. Additionally, this allowed the rumen to adjust to the new cationic close-up dry ration (CUD; Table 9). Daily feed was offered once in the morning (0700-0900 h) and then in the afternoon (1300-1500 h). The morning feeding was set at ≅13.5 kg of TMR per cow and contained daily

Table 9. Percentage of constituents and composition of total mixed rations (TMR) fed to Holstein cows supplemented with glucogenic precursors, dry matter basis[1].

	Feeding period		
Composition	Prepartum	Postpartum[2]	
Ingredients (%)		Minimum	Maximum
Corn Silage	51.70	13.10	28.00
Sorghum Silage	----	0.00	13.10
Alfalfa Hay	4.90	9.30	13.00
Cottonseed Hulls	6.50	4.50	6.40
Citrus Pulp	6.50	8.60	10.80
Corn Meal	14.20	18.20	22.90
Soybean Meal	7.00	5.10	11.80
Whole Cottonseed	0.70	0.00	12.90
Mineral Mix	1.30	3.70	4.00
Prolac	----	0.00	2.10
Soyplus	7.80	0.00	8.50
Oat Silage	----	0.00	6.00
Molasses	----	0.00	3.20
Nutrients			
DM (%)	46.40	51.68	62.79
NEL (Mcal/kg)	1.64	1.58	1.70
CP (%)	14.66	17.62	18.23
RUP (%)	5.59	6.14	6.62
RDP (%)	9.07	11.21	11.99
NDF (%)	34.04	29.38	35.49
ADF (%)	22.08	20.66	25.26
DCAD (meq/kg)[3]	21.28	265.64	377.42

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[1] All treatment cows received the same total mixed ration (TMR) as control plus their respective glucogenic supplement. Treatments were: CON= no supplement; NUT= 113 g/d of NutroCal; MET= 454 g/d of Metaxerol; PPG = 300 mL/d of propylene glycol

[2] Postpartum diet changed slightly throughout the experimental period. Minimum, maximum and average content of each ingredient used for all different diets used was estimated.

[3] DCAD=(%K x 256 + %Na x 435) - (%Cl x 282 + %S x 624).

allotment of supplement. The afternoon TMR contained no glucogenic precursor and the amount offered was set to allow for approximately 5% total daily weigh back. Daily feed intake and weigh back were recorded. The goal of this strategy was to ensure essentially full consumption of the daily allotment of precursor fed and to better mimic commercial feeding practices where the precursors would be top-dressed to the TMR. After calving, cows were moved to an adjacent free-stall barn equipped with Calan Gates. At time of move cows were fed the lactating herd TMR (Table 9) and appropriate precursors were included in the a.m. feed allotment (none, NUT, MET or PPG). The same 2X/d feeding strategy used prepartum was continued through d 28 postpartum. On d 29 postpartum, all cows were moved to another free-stall barn with the milking herd and fed the same TMR but addition of glucogenic precursors was discontinued.

Blood Collection, Handling , Storage and Analyses

Blood samples were collected from the coccygeal vein of all cows three times weekly before the a.m. feeding or milking (0600-0800 h) using Vacutainer® needles (2.54 cm) and 10x100 mm tubes containing sodium heparin (Becton-Dickinson, Fairlawn, NJ). They were placed in an ice-water bath immediately after collection, centrifuged within 3 h at 3000 RPM (2,619 xg) for 30 min at 5 °C (Jouan GR 412 centrifuge, Winchester, VA), and plasma was collected and stored in capped polypropylene tubes at -20 °C until analyzed.

Double antibody radioimmunoassay procedures were used to determine plasma concentrations of IGF-I, after extraction (Enright, 1989), by method of Abribat et al. (1990) and INS by method of Malven et al. (1987). An enzymatic colorimetric procedure (NEFA C, Wako Pure Chemical Industries, Osaka, Japan) was used for quantitative determination of NEFA in plasma as described by Johnson and Peters (1993). Glucose was analyzed using a glucose oxidase procedure (Kit 510, Sigma diagnostics, St.Louis, MO) as described by Raabo and Terkildsen (1960). Concentrations of β-HBA were determined using the reagent set developed by Pointe Scientific, Inc (anonymous). Glucose, NEFA and β-HBA assays were carried out in 96-well microtiter plates.

Measurements

Body condition scores and body weights

Body condition score (1-5, thin to fat; Ferguson et al., 1994) and BW of cows were recorded on the same day each week (0730 to 0900 h) before a.m. feeding or milking. Measurements began on d -21 (prepartum) through d 70 postpartum and thereafter measurements were biweekly through 150 d postpartum.

Milk yield and composition

Daily milk production was recorded at each of the three daily milking shifts through 150 DIM and milk samples were collected at each of these milking shifts once a week but only through 70 DIM. Milk samples were preserved using spectrum Microtab™ preservative (D&F control systems, Inc.)

and analyzed for contents of fat, protein, and somatic cells (SCC) at DHI laboratory (Southeast Dairy Lab, McDonough, GA).

Non-esterified fatty acid (NEFA) assay

The methodology employed was as described by Johnson and Peters (1993). Two reagents were prepared and used as follows: reagent A was prepared by adding 10 mL of dilution solution A to reagent A bottle and mixed with 13.3 mL of 50 m*M* phosphate buffer. Reagent B was prepared by adding 20 mL of dilution solution B to reagent B bottle and mixed with 33.3 mL of 50 m*M* phosphate buffer. Five standards were prepared to give 0, 200, 400, 600 and 800 µEq/L as follows:

Standard (µEq/L)	Stock Solution (µL)	0.9% Saline Solution (µL)
0	0	500
200	100	400
400	200	300
600	300	200
800	400	100

Samples, standards and controls were mixed before being transferred into microtiter wells. Five microliters (5 µL) of plasma, standards and controls were transferred to each of the corresponding wells, followed by 100 µL of color reagent A to each. Plates were incubated in a forced air oven at 25°C for 30 min. After this incubation 200 µL of color reagent B were added to each well followed by another incubation period of 30 min at the same temperature.

After this second incubation period, plates were allowed to sit for 5 min on the bench at room temperature and then were read in an Automated Microplate Reader (Model EL 309, Bio-tek Instruments, Inc., Laboratory division, Winooski, VT) using a blank as reference at 550nm wavelength. The equation of the standard curve was calculated and sample NEFA concentrations calculated in an Excel spread sheet. All samples were analyzed in duplicate and standards in triplicate. Any sample with ≥ 7% error was assayed again.

Glucose assay

An enzymatic method modified by Raabo and Terkildsen (1960) was used for assay of glucose in the plasma samples. Concentrations of glucose were determined using a Sigma Diagnostics Glucose Enzymatic kit (# 510) purchased from Sigma Chemical Co. (St. Louis, MO). Specific concentrations of standards used were: 0, 25, 50, 75, and 100 mg of glucose/dL. These were prepared by diluting the glucose standard solution provided (100 mg/dl) with distilled water to achieve the desired concentrations. Standard dilutions used in the assay were as follows:

Standard	Stock Solution (μL)	Water (mL)	5% Zinc Sulfate (mL)	0.3 N Barium Hydroxide (mL)
0	0	1.0	0.500	0.500
25	25	0.975	0.500	0.500
50	50	0.950	0.500	0.500
75	75	0.925	0.500	0.500
100	100	0.900	0.500	0.500

The enzyme solution was prepared by dissolving one capsule of PGO in 100 mL of distilled water. The color reagent was prepared by mixing the contents of one vial of o-dianisidine dihydrochloride and 20 mL of distilled water. One hundred milliliters (100 mL) of the enzyme solution were mixed with 1.6 mL of color reagent. One hundred microliters of plasma were added to borosilicate tubes that contained 900 µL of distilled water. All sample tubes were prepared in duplicate. The precipitation of proteins was effected by adding 500 µL of barium hydroxide solution (0.3N) and 500 µL of zinc sulfate solution (5%) into all tubes, including standards prepared the same way as samples. The tubes were vortexed and centrifuged at 2500 RPM (1,819 xg) for 20 min at 4°C (RC-3B, refrigerated centrifuge, Sorvall Instruments).

Twenty-five microliters of each supernatant sample and standard were added to wells in a 96-well (0.45 mL well capacity) polypropylene plate (Fisher Scientific, Pittsburgh, PA) then 225 µL of combined enzyme-color reagent solution [(1 capsule of PGO enzymes + 1.6 mL o-dianisidine dihydrochloride - color reagent solution) + 100 mL of distilled water] were added. Standards were assayed in triplicate and samples in duplicate (two wells for each sample tube which were prepared in duplicate for each sample). The plasma protein-free supernatant and combined reactants were incubated at room temperature for 45 min. After incubation, the absorbance was read in an Automated Microplate Reader (Model EL 309, Bio-tek Instruments, Inc., Laboratory division, Winooski, VT) using water as reference at 450 nm wavelength. Linear regression of absorbance and glucose concentration of the glucose standards was used to determine the concentration (glucose/dL) in unknown plasma samples. Final concentrations were expressed as mg/dL.

Calcium assay

Calcium determination was conducted following the procedure of Miles et al. (2001). Reagents for this procedure were the following: 5% lanthanum oxide (117.3 g La_2O_3 in 500 ml HCl; after reaction subsided, volume was brought to 2 L with deionized water), 50% trichloroacetic acid (TCA; 500 g TCA/bottle was brought to 1 L by adding deionized water), precipitation mixture containing 1% lanthanum and 10% TCA (200 mL of 5% La and 200 mL of 50% TCA were added to a 1000 mL volumetric flask and brought to volume with deionized water), and Ca stock solution was used as a reference solution (1,000 ppm; Fisher SC191-500) diluted to 100 ppm (1mL Ca + 9 mL deionized water). Standards were prepared in 100 mL volumetric flasks and then brought to volume with deionized water as described as follows:

	Std 0	Std 5	Std 10	Std 15
100 ppm Ca Stock (mL)	0	5	10	15
50% TCA (mL)	18	18	18	18
5% La (mL)	18	18	18	18

In 5 mL polypropylene tubes (12x75 mm), 500 µL of plasma sample were added followed by 4.5 mL of precipitation mixture for a total volume of 5 mL. A set of water samples also were prepared (500 µL of water + 4.5 ml precipitation mixture). All tubes were vortexed and then centrifuged for 20 min at 2,500 RPM (1,819 xg) at 4°C (RC-3B, refrigerated centrifuge, Sorvall Instruments). Supernatant was transferred to a second set of polypropylene tubes (12x75 mm), which were capped and kept refrigerated until Ca concentration was determined by flame atomic absorption spectrophotometry.

β-hydroxybutyrate (β-HBA) assay

The methodology employed was as described in Pointe Scientific Inc., Reagent set flyer (2003). Two reagents are used in this procedure. Reagent 1 (R1) contained β-hydroxybutyrate dehydrogenase and Diaphorase enzymes. Reagent 2 (R2) contained NAD and a reduced color reagent (INT). Both reagents were prepared by Pointe Scientific and were used in the assays as received. A working reagent (RW) was prepared by mixing 10 parts of R1 with 1.5 parts R2. Three standards provided by Pointe Scientific at concentrations of 0.2, 1.0, and 4.0 m*M* β-HBA were used to establish the standard curves.

Sample, standards and controls were mixed before being transferred into microtiter wells. Three microliters (3 µL) of plasma, standards and plasma control were transferred to each of the corresponding wells, followed by 120 µL of RW to each well. Plates were incubated in a forced air oven at 37°C for 5 min. After this incubation period, the absorbency was read in an Automated Microplate Reader (Model EL 309, Bio-tek Instruments, Inc., Laboratory division, Winooski, VT) using a 490 nm wavelength filter. Values were entered into an Excel spread sheet, a regression curve calculated for the β-HBA standards, and sample concentrations of β-HBA calculated. All samples were analyzed in duplicate and standards in triplicate. Any sample with 7% error or greater was assayed again. Final concentrations were expressed as mg/dL.

Liver Biopsies, Samples and Analyses.

A sub-sample of 40 cows (10 cows per treatment) was assigned to be biopsied at 3 wk before expected calving (-3), at calving, and at 2 and 4 wk postpartum. For biopsy, cows were immobilized in a squeeze chute and the hair on their right side was clipped, from approximately 8-14 inches from top of ribs over the last four ribs. A local anesthetic (Lidocaine) was used at the site where the incision was to be made. Surgical preparation included clipping hair, scrubbing area with Betadine surgical scrub, and rinsing this area with sterile water; this procedure was repeated three times. The surgical field then was rinsed with 70% ethyl alcohol and then injected the Lidocaine (10 ml of 2% solution of Lidocaine hydrochloride as the anesthetic) in between the last two or three ribs on the right side of the cow along a line extending from hip joint to the elbow of the front leg. The surgical area then was rinsed with iodine (7% strong tincture) and wiped clean with sterile gauze. The surgical incision was made (~1.25 cm long) and a sterile biopsy tool used to penetrate the peritoneal tissue and the liver was located by palpation. The biopsy plunger then was forced into the liver along with the biopsy tool, and the plunger then was retracted out of the biopsy tool at the same time a clean 50 ml syringe was used to apply a slight vacuum (approximately 1/3 draw of the full syringe) to the tool body to secure and retain the liver tissue in the tool body. The tool was removed from liver and the liver tissue recovered was expelled into a beaker of sterile saline (0.9% NaCl), washed free of blood, blotted on filter paper, placed in a small labeled polystyrene plastic vial, and flash frozen in liquid nitrogen. The surgical site was closed with two to three surgical staples. No general antibiotics were required but the surgical site was sprayed with an antibiotic spray at the conclusion of the surgery. The cow and surgical site were observed for up to 2 h after surgery for any signs of distress while either retained in the squeeze chute or in her free-stall barn and then during several days. The staples used to close the surgical site were removed after approximately 7-10 d. The time to complete preparation and actual biopsy procedure on each cow was approximately 15 min. There were no adverse effects or complications from the biopsy procedure, as determined by daily monitoring of the animal during both the prepartum and postpartum periods.

Liver total lipid determination. For determination of total liver lipids two sets of glass screw-cap tubes were numbered and dried overnight at 105°C in a forced air oven. One set of tubes (25 ml screw-cap glass tubes) was weighed to the fourth decimal place without caps for two consecutive days and weights were recorded. These tubes were stored in a desiccator until needed. One hundred milligrams of freshly thawed liver sample were placed into 25 mL screw-cap glass tubes followed by addition of 10 mL of chloroform:methanol (2:1) solution. All tubes were vortexed and samples were homogenized for 30 sec using a Polytron tissue homogenizer. Tubes were placed on a horizontal shaker for 5 min after which 4 mL of distilled water were added to all tubes and they were vortexed. Tubes were centrifuged for 5 min at 1,700 RPM (900 xg). The top layer (water-methanol) was removed by aspiration and discarded using transfer pipets. The remaining mixture in the tube (chloroform:methanol and liver tissue) was poured through filter paper (Whatman 425 mm diameter; Cat.# 1822042) placed inside the Buchner funnel and liquid was collected into the pre-weighed glass tubes. The extraction tube was rinsed three times with 3 mL of chloroform and this was poured through the funnel and collected in the glass tubes. All tubes were placed in a rack, set in a water bath at 40-50°C and dried under a stream of air for about 30 min. The rack of tubes was transferred into the forced air oven (80°C) until dried, then placed in a desiccator, and re-weighed. Total lipid was calculated by weight difference and lipids were expressed as percentage of wet weight of the original liver sample.

Radioimmunoassays

Double antibody radioimmunoassay procedures were used to measure IGF-1 and insulin concentrations in plasma. All samples from individual cows were assayed in duplicate in single assay.

Second antibody preparation

Two second antibodies were prepared using Florida Native sheep managed at the DRU. These antibodies were used in the radioimmunoassay of IGF-I and insulin. Rabbit gamma globulin and guinea pig gamma globulin were injected into sheep to prepare these antibodies. Either 20-25 mg guinea pig gamma globulin or 30-40 mg rabbit gamma globulin were placed in 25 mL Erlenmeyer flasks, 5 to 15 mL of distilled water were added and stirred on a magnetic stirrer plate. Equal amounts of Freund's complete adjuvant were added and mixed in micro-blender to the consistency of whipped cream (about 1 min on high speed). About 5 mL of this mixture was injected subcutaneously (SC) into individual sheep over the shoulder on both left and right sides in three or four different sites. After 10 to 14 d, the same procedure was repeated except that the gamma globulins were mixed with Freund's incomplete adjuvant. After an additional 2 wk wait, sheep were bled from the jugular vein; 400 mL of blood were collected using a butterfly needle and 60 mL syringe to withdraw the blood. All blood was placed in 40 mL screw-top centrifuge tubes, capped and placed on ice as soon as possible and then left overnight (16 h at 4^{o}C). Tubes containing the clotted blood were centrifuged at 5000 RPM (7,277 xg) for 30 min in the RC-3B centrifuge. Serum obtained was pooled and frozen. Sheep were bled again after an additional 4 wk. Sheep were reinjected with gamma globulin mixed with Freund's incomplete adjuvant, as described, each 2 to 3 mo and in some cases up to 6 mo elapsed between bleedings. These pools of sheep anti-rabbit (SAR) and sheep anti-guinea pig (SAGP) sera were used for assays as the second antibody.

Insulin-like growth factor 1 (IGF-I)

A double antibody radioimmunoassay procedure, as described by Abribat et al. (1990) and modified for sample extraction by Enright et al. (1989) and Daughaday et al. (1980) was used for IGF-I determinations.

Extraction of IGF-I from binding proteins. A mixture of ethanol: acetone:acetic acid (EAA 60:30:10, by volume) was used for extraction.

Exactly 400 µL extraction mixture were added to 100 µL plasma in 12 x 75 mm polystyrene tubes, they were mixed thoroughly for 15 sec using a vortex and allowed to stand for 30 min after mixing. Tubes were centrifuged at 3000 RPM (2,619 xg) for 30 min in a refrigerated centrifuge (RC-2B). Two hundred and fifty µL of the supernatant were transferred to 12 x 75 mm polystyrene tubes, then 100 µL of 0.855 *M* trizma base were added. After that 350 µL of the assay buffer were added to make the final dilution 1:140.

Procedure. Highly purified human insulin-like growth factor 1 (IGF-I), supplied by Upstate Biotechnology Incorporated (Richmond, CA), was dissolved (0.5 µg/µL) in 0.1 *M* acetic acid (pH=2-5), and 10 µL aliquots were transferred to 1 mL microfuge vials, capped and frozen to be used for the iodination procedure.

Highly purified bovine insulin-like growth factor 1 (IGF-I); supplied by Monsanto Company (St Louis, MO) was dissolved (10 µg) in 100 µL of 0.1 M acetic acid to give stock 0 (100 ng/mL), and then aliquoted into tubes (10 µL/ tube) and frozen. To prepare stock 1, 10 µL of stock 0 were added to 490 µL of assay buffer. Stock 2 was prepared by adding 10 µL of stock 1 to 990 µL of assay buffer; this had final concentration of 20 pg/mL. From stock 2, standards were prepared to contain 5, 10, 20, 40, 60, 80, 100, 150, 200, 300, 400 and 500 pg IGF-I/mL. The first antibody (primary), rabbit anti-bovine IGF-I, was obtained as a gift from Drs. Louis Underwood and Judson J. Van Wyk, Division of Pediatric Endocrinology, University of North Carolina, Chapel Hill. It was dissolved (1:160,000) in assay buffer (200 mg/L protamine, 4.4 g/L sodium monobasic phosphate, 10 mL of 2% of sodium azide, 3.72 g/L EDTA (0.013 M) and 2.5 g/L BSA). The second antibody, sheep anti-rabbit gammaglobulin (SAR), was prepared, as described and was diluted 1:4 in EDTA-PBS.

Plasma sample extracts were used in the assay, (10 µL of plasma extract and 190 µL assay buffer). Greater or lesser quantities of sample extract were used if the resulting growth factor concentration in the sample was very low or very high and outside the range of prepared standards. Immediately thereafter, 100 µL iodinated IGF-I and 100 µL diluted primary antiserum were added to all tubes, except total count and nonspecific binding tubes. After incubation for 24 h at 4 ^{0}C, diluted second antibody (100 µL) SAR and 50 µL normal rabbit serum (1:50) were added to all tubes except the total count tubes and allowed to stand 30 min. After that, 1 mL of 6% polyethylene glycol (PEG) in assay buffer was added, vortexed and allowed to stand for 15 min, then tubes were centrifuged at 3000 RPM (2,619 xg) for 30 min at 4 ^{0}C (RC-3B, refrigerated centrifuge, Sorvall Instruments), decanted and inverted on absorbent paper to drain. After tubes were dry, bound radioactivity in tubes was measured using a Packard^R auto gamma counter (model B-5005). Final results were calculated using the spline radioimmunoassay data processing procedure.

Iodination and protein separation. One vial, containing 10 µL IGF-I (0.5 µg/µL), was thawed 1 h prior to iodination, then 10 µL 0.01 *M* phosphate buffer were added to the thawed aliquot. The column used to separate the iodinated protein from free iodine was prepared by cutting off the mouthpiece of a 10 mL disposable glass pipette. A small glass bead or glass wool plug was placed into the column before adding the Sephadex column packing (Sephadex G-50, Sigma Chemical; dispersed in 0.01 *M* phosphate buffer) until the Sephadex filled the column. Subsequently, the column was washed with 2 mL 0.5 M phosphate buffer that contained 1.0% BSA followed by 10 mL 0.01 *M* phosphate buffer. Buffer (0.01 *M*) was retained at the top of the column to cover Sephadex packing until used for protein separation.

Immediately prior to the iodination, chloramine-T (3 mg/mL) and sodium metabisulfate (5 mg/mL) each were dissolved in 1 mL phosphate buffer. Sequentially, 0.5 *M* phosphate buffer (20 µL), radioactive iodine (I^{125} ;10 µL 0.5 mci.) and 10 µL chloramine-T were added to the vial that contained IGF-I. After a reaction time of 25 sec, with mixing, 10 µL sodium metabisulfite and 30 µL 0.01 *M* phosphate buffer were added to the vial to stop the reaction. This solution was transferred to the top of the Sephadex column then the reaction vial was rinsed with 50 µL 0.01 M phosphate buffer to ensure complete transfer of iodinated protein.

Borosilicate tubes (15 x 100 mm) numbered 1-50 were filled with 500 µL 0.5 M phosphate-BSA buffer and placed in a fraction collector to receive eluate (20 drops) from the Sephadex column. Aliquots (10 µL) of each eluted fraction were transferred to a second set of tubes for use in identifying elution peaks by counting radioactivity using a Tracor Analytic Gamma counter (Nuclear-Chicago, Gamma Trac 1191, G. D. Searle and Co., Des Plaines, IL). Collection tubes that contained the first large peak, which corresponded to I^{125}

bound to protein, were pooled. The second large peak represented free I^{125}. The iodinated IGF-I was stored at 4 °C and used within 10 d of preparation.

Insulin assay

A double antibody radioimmunoassay procedure, as described by Soeldner and Sloane (1965) and modified by Malven et al. (1987), was used for assay of insulin in plasma samples. Highly purified insulin (Sigma Immunochemicals, St. Louis, MO) was weighed (≈100 µg) as stock insulin and dissolved in 30 mM HCL (pH=2.5) using an ultra sonic water bath. The insulin then was aliquoted (10 µL) into 1 mL microfuge vials and frozen to be used for the iodination procedure. The stock insulin was diluted in assay buffer (borate/BSA; 0.133 M borate, 0.01% merthiolate, with 0.5% BSA) to give a final concentration of 100 ng/mL for preparation of standards which contained 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 ng insulin/mL. The first antibody (primary), guinea pig anti-bovine insulin, was obtained from Sigma Chemical Co.(St. Louis, MO). It was dissolved in borate/BSA buffer (1:20,000). The second antibody, sheep anti-guinea pig gammaglobulin, was prepared, as described and was diluted 1:4 in borate/EDTA assay buffer.

One hundred fifty microliters of plasma were used for assay. Samples were prepared and assayed in duplicate after dilution with 100 µL assay buffer in 12 x 75 mm borosilicate tubes. Immediately thereafter, 100 µL diluted primary antiserum were added to all tubes. Six hours later 100 µL of iodinated insulin (≅ 30,000 CPM) were added to all tubes. After incubation for 24 h at 4°C, diluted second antibody (100 µL) SAGP (1:4 dilution) and 100 µL of

NGPS (1:200 dilution) were added to all tubes except the total count tubes, and incubated for 10 min. After that, 750 µL of 15% polyethylene glycol (PEG) in borate buffer were added to all tubes except the total count tubes, and tubes were mixed using a vortex. The tubes were centrifuged at 3000 RPM (2,619 xg) for 30 min at 4 ^{0}C (RC-3B, refrigerated centrifuge, Sorvall Instruments), decanted, and inverted on absorbent paper to drain and dry. After tubes were dry, bound radioactivity in tubes was measured using a Packard^R auto gamma counter (model B-5005). Final results were calculated using the spline radioimmunoassay data processing procedure.

Iodination and protein separation. One vial containing 10 µL insulin (0.5 µg/µL) was thawed 1 h prior to iodination, then 10 µL of 0.5 M phosphate buffer were added. The column used to separate the iodinated protein from free iodine was prepared by cutting off the mouthpiece of a 10 mL disposable glass pipette. A small glass bead or glass wool plug was placed into the column before adding the Sephadex packing (Sephadex G-50, Sigma Chemical; dispersed in 0.01 *M* phosphate buffer) until the Sephadex filled the column. Subsequently, the column was washed with 2 ml 0.5 *M* phosphate buffer that contained 0.5 % BSA followed by 10 mL 0.01 *M* phosphate buffer. Phosphate buffer (0.01 *M*) was retained at the top of the Sephadex bed until it was used for protein separation.

Immediately prior to the iodination, 3 mg of chloramine-T and sodium metabisulfate (5 mg/mL) were weighed then each was dissolved in 1 mL 0.5 *M* phosphate buffer. Sequentially, 0.5 *M* phosphate buffer (20 µL), radioactive iodine (I^{125}, 1 mci, 10 µL) and 10 µL chloramine-T were added to the vial that contained the insulin. The reaction lasted 15-45 sec, during which contents were mixed by finger tapping, then 10 µL of sodium metabisulfite were added to stop the reaction and 30 µL 0.01 *M* phosphate buffer were added to the vial. This solution was mixed and transferred to the top of the Sephadex column, then the reaction vial was rinsed with 50 µL 0.01 *M* phosphate buffer to ensure complete transfer of the iodinated protein.

Borosilicate tubes (15 x 100 mm) numbered 1-40 were filled with 500 µL 0.5 *M* borate-BSA buffer. Tubes were placed in a fraction collector to receive eluate (20 drops) from the Sephadex column. Aliquots (10 µL) of each eluted fraction were transferred to a second set of tubes for use in identifying elution peaks by counting radioactivity in a Tracor Analytic Gamma Counter (Nuclear-Chicago, Gamma Trac 1191, G. D. Searle and Co., Des Plaines, IL). Collection tubes that contained the first large peak, which corresponded to I^{125} bound to protein, were pooled. The second large peak was free I^{125}. The iodinated insulin was stored at 4 ^{0}C and used within 10 d of preparation.

Statistical Analysis

Data were analyzed by proc GLM least squares analyses of variance (SAS, 1993) and proc MIXED methods of SAS (Littell et al., 2000). The model used included main effects of TRT, SEA, the two-way interaction, and the residual error term. Cow nested within the interaction of TRT by SEA was used as the random variable. All analyses, except for milk yield, were conducted separately for prepartum and postpartum periods. Cows BW and BCS at the time they entered the experiment, or their calving BW and BCS were used as covariates as appropriate. Differences of least squares means (PDIFF) obtained from GLM and MIXED methods of SAS were used to determine differences between means. Orthogonal contrasts were used to compare 1) CON vs. NUT, MET, PPG; 2) NUT, MET vs. PPG; 3) NUT vs. PPG. Residual correlation analyses (MANOVA) were used to examine association among plasma hormones, growth factor, and metabolites analyzed in the current study. Model used was: Y= TRT SEA TRT*SEA COW(TRT*SEA) DAY DAY^2 DAY^3.

Results and Discussion

It is well established that DMI decreases as calving approaches. The DMI can decrease from 2% of BW during the first few weeks of the 60-d dry period to ~1.4% of BW during the 7-10 d period before calving. This ~30% decrease normally occurs rapidly during the latter days of the prepartum portion of the transition period (Bertics et al., 1992; Ingvartsen and Anderson, 2000; Overton, 2001). During the first 3 wk post-calving DMI typically increases at the rate of 1.5 to 2.5 kg/wk (Grant and Albright, 1995) with the increase being more rapid in multiparous cows (Kertz et al. 1991).

These expected changes in DMI by cows have been attributed to the physiological changes they undergo during the transition period and especially the changes in specific hormones. For example, plasma insulin and IGF-I continuously decline during the prepartum phase of the transition period until calving, whereas concentrations of ST increase rapidly between the end of gestation and the initiation of lactation. Concentrations of progesterone (P_4), which are high during gestation, decrease rapidly as calving approaches and are very low at calving. In addition, there are transitory increases in concentrations of estrogen (E_2) and glucocorticoids during the periparturient period. These hormonal changes not only contribute to the observed decline in DMI, but they also are an integral part of the process of coordinated metabolic changes that herald the onset of parturition and that favor the mobilization of body fat reserves from adipocytes (Grummer, 1995).

Prepartum statistical analyses for DMI, BW, and BCS were adjusted for the initial BW and BCS at the time cows entered the experiment. Cows in the current study showed the expected pattern in DMI during the transition period (Figures 8 and 9). Least squares means for DMI are in table 10. The decrease in DMI from -3 wk to the day of calving was ~24.3% (from 13.15 to 9.95 kg/d). This certainly was within the range of values reported by various researchers (Kertz et al., 1991; Bertics et al., 1992; Grant and Albright, 1995). Although the decrease was similar to that seen in many other studies, no significant differences were detected among treatments. Therefore, including glucogenic compounds in the prepartum close-up diet did not alter DMI or the expected decline.

Season of calving effects on DMI were detected during the prepartum period (Figure 9; Table 10); cows that calved during the cooler months (SEA 1) had lesser DMI compared to cows that calved during SEA 2 ($P<0.0001$). During the prepartum period, TRTxSEA interaction also was detected

Table 10. Least squares means and SE for DMI, BW and BCS of Holstein cows fed glucogenic supplements during the transition period (-3 wk to + 4 wk).

	DMI (kg/d)[1]				BW (kg)[2]				BCS[2]			
Treatment[3]	PRE[4]	±SE[4]	POST[4]	±SE[4]	PRE[4]	±SE[4]	POST[4]	±SE[4]	PRE[4]	±SE[4]	POST[4]	±SE[4]
CON	13.3	0.31	18.6^{b}	0.32	741.5^{b}	3.9	654.2^{a}	5.4	3.6	0.038	3.14^{ab}	0.052
NUT	13.3	0.30	17.8^{a}	0.30	740.5^{b}	3.7	661.3^{ab}	5.1	3.6	0.036	3.23^{b}	0.049
MET	13.0	0.31	18.5^{ab}	0.31	740.0^{b}	3.7	670.3^{b}	5.4	3.5	0.037	3.07^{a}	0.05
PPG	12.7	0.30	18.1^{ab}	0.32	731.4^{a}	3.7	667.8^{b}	5.5	3.6	0.036	3.23^{b}	0.051
Season[5]												
SEA 1	12.3^{a}	0.24	17.5^{a}	0.24	737.3	2.7	662.7	3.9	3.6^{b}	0.027	3.19	0.037
SEA 2	13.9^{b}	0.20	19.0^{b}	0.20	739.2	2.6	663.6	3.6	3.5^{a}	0.025	3.15	0.034

[1] DMI= dry matter intake. Recorded daily from 3 wk prepartum through 4 wk postpartum.

[2] BW=body weight; BCS=body condition score. Recorded weekly from 21 d prepartum through 70 d postpartum, thereafter biweekly until 150 DIM.

[3] CON= no supplement; NUT= 113 g/d of NutroCal; MET= 454 g/d of Metaxerol; PPG = 300 mL/d of propylene glycol fed from 3 wk prepartum to 4 wk postpartum.

[4] PRE= prepartum (-3 wk to calving); ±SE= plus or minus standard error; POST= postpartum (calving to +4 wk).

[5] SEA 1= cooler (October-March); SEA 2=warmer (May-September).

[ab] Means with different letters within columns within treatment or within season differed ($P<0.05$;PDIFF).

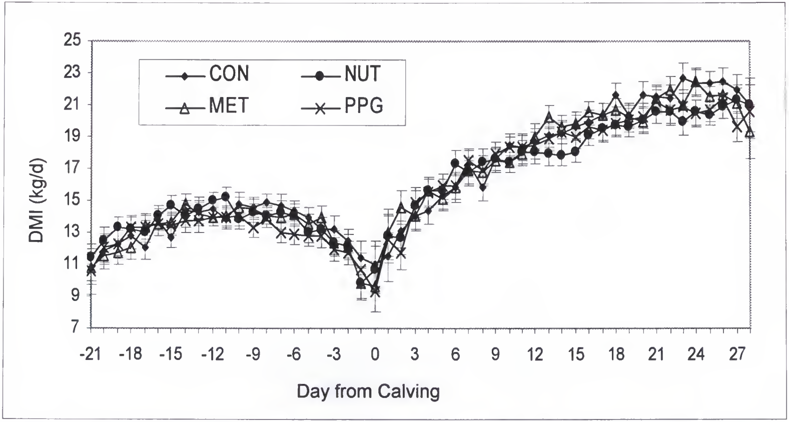

Figure 8. Least squares means and SE for dry matter intake (DMI) from 3 wk prepartum through 4 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds. Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol.

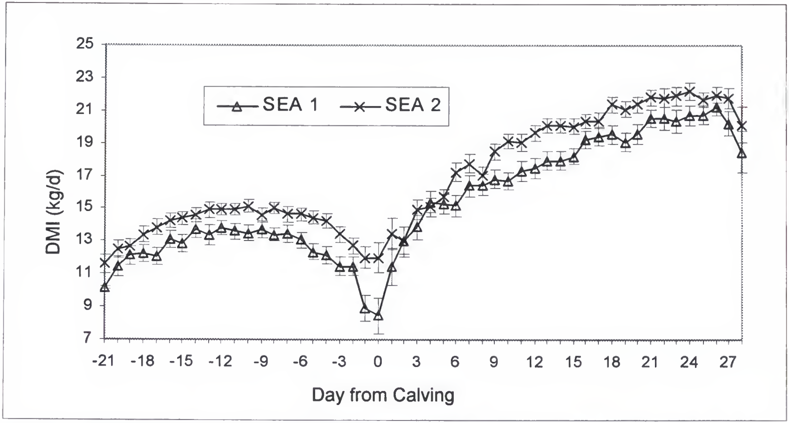

Figure 9. Least squares means and SE for dry matter intake (DMI) from 3 wk prepartum through 4 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds during two seasons. Seasons were: SEA 1= cooler (October-March); SEA 2=warmer (May-September). Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol.

(P=0.0085). Cows that calved during SEA 2 had greater DMI (1.66, 3.2, 0.66, 0.74 kg/d for CON, NUT, MET, PPG, respectively) within each treatment group.

The prepartum increases in BW and BCS seen doubtless reflected the growth of the calf and the sustained high level of DMI throughout most of the 3 wk prepartum until the decline seen shortly before calving (Figures 10-13). No significant differences for BW or BCS were detected among treatments or season of calving during the prepartum period . Least squares means for BW across treatments and seasons of calving are in table 10. Initial BW of cows supplemented with PPG tended to be less compared to cows on the other three treatments ($P \leq 0.0943$). This group tended to gain weight at a lower rate compared to the cows in other treatment groups and remained lower throughout the prepartum period (Figure 10). Orthogonal contrasts (Appendix Table A) showed that PPG cows weighed less during the prepartum period compared to cows supplemented with NUT and MET (P=0.05; 731.4 vs 740.5 and 740.0 kg for PPG, NUT and MET, respectively). Additionally, BW means of control cows and cows supplemented with PPG tended to differ (741.5 vs. 731.4 kg for CON and PPG, respectively; P=0.0744). No effect of season of calving was detected for BW. Least squares means for BW were 737.3 and 739.2 kg for SEA 1 and SEA 2, respectively (Table10). Mean initial BCS of cows when assigned to experiment were 3.4, 3.5, 3.4, and 3.4 for CON, NUT, MET, and PPG. Cows in current experiment maintained similar BCS throughout the prepartum period (means were 3.6, 3.6, 3.5 and 3.6 for CON, NUT, MET, and PPG) and no treatment effect was detected (Table 10). No

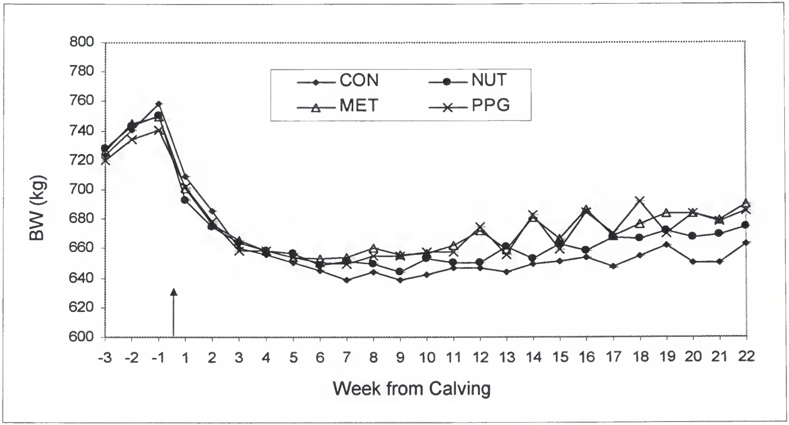

Figure 10. Least squares means and SE for body weight (BW) from 3 wk prepartum through 22 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds. Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 ml/d propylene glycol. Arrow indicates calving.

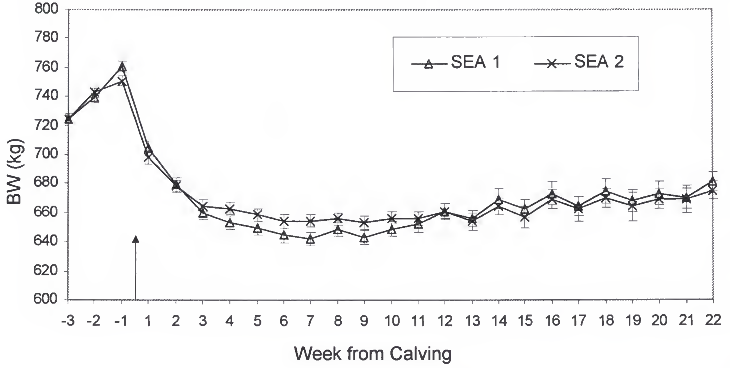

Figure 11. Least squares means and SE for body weight (BW) from 3 wk prepartum through 22 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds during two seasons. Seasons were: SEA 1= cooler (October-March); SEA 2=warmer (May-September). Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Arrow indicates calving.

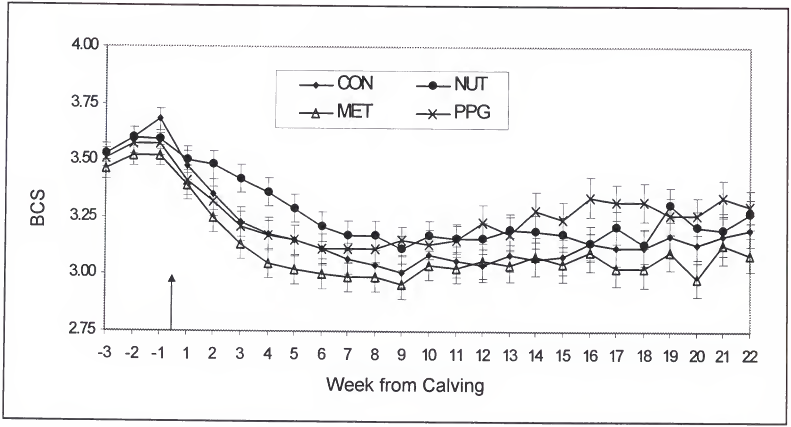

Figure 12. Least squares means and SE for body condition score (BCS) from 3 wk prepartum through 22 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds. Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Arrow indicates calving.

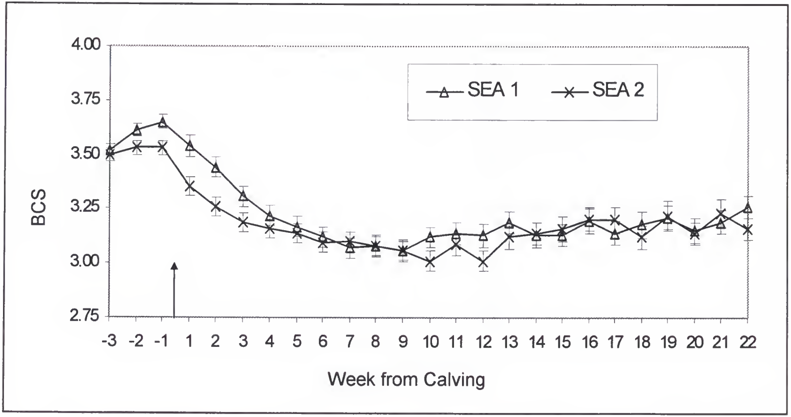

Figure 13. Least squares means and SE for body condition score (BCS) from 3 wk prepartum through 22 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds during two seasons. Seasons were: SEA 1= cooler (October-March); SEA 2=warmer (May-September). Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Arrow indicates calving.

SEA effect was detected on BW, whereas BCS for cows in SEA 1 was greater compared to SEA 2, during the prepartum period. Orthogonal contrasts for BCS were conducted to test 1) CON vs. NUT, MET, and PPG, 2) PPG vs. NUT and MET, and 3) NUT vs. MET. No significant differences were detected for any of the contrasts ($P > 0.10$) during the prepartum period (Appendix Table A).

The rapid increase in milk production after calving requires cows to mobilize fatty acids, protein, and minerals to meet their requirements for energy, fatty acids, amino acids, and minerals to support maintenance, growth, if still occurring in younger animals, and the rising demands of lactation. This tissue mobilization leads to the expected decrease in BW and BCS. Overall, loss of BW and BCS continues through peak lactation after which cows start to gain weight as DMI increases and energy balance becomes positive. It is widely accepted that high producing cows also lose BCS due to the negative energy balance at the beginning of lactation. Many studies have evaluated and demonstrated the relationship among BCS and BW at calving and the subsequent DMI, productivity, reproductive capacity and health of cows at the beginning of lactation. Heavier cows have greater DMI but the proportion of DMI to BW tends to be similar to lighter cows. Current recommendations are to have modern dairy cows attain a BCS between 3.25 and 3.75 at the time they are dried-off and that they should maintain that score, or at least not increase more than a quarter point during the dry period. Cows that are too heavy and have too great a BCS tended to increase postpartum DMI at a slower rate after calving (Hayirli et al., 2002). This would be detrimental to milk production.

Statistical analyses for postpartum DMI, BW, and BCS were adjusted for calving BW and BCS (BW and BCS taken within 12 h after calving). In the current study, approximately 10% of cow BW was lost due to the expulsion of the calf, placenta and fluids. This accounted for the dramatic decline in BW seen across treatments (Figures 10 and 11). At this time DMI still was low (Figures 8 and 9) but did began to increase as the demand for energy and nutrients increase to support the lactation. Although the decrease in DMI after calving in the current study was slightly greater than 24% around time of calving, cows on the four treatments showed a rapid increase in DMI from 10.12 kg/d at calving to 12.44, 14.45, 15.53 and 17.12 kg/d on days 1, 3, 5 and 7 postpartum. Average DMI across treatments for the postpartum period was 18.25 kg/d. This represented an increase in DMI of 38% relative to calving day DMI. No treatment effect on mean DMI was detected during the first 28 d of the postpartum period during which DMI measured. Overall least squares means for DMI during the 4 wk postpartum period were 18.6, 17.8, 18.5, and 18.1 kg/d for CON, NUT, MET, and PPG, respectively (Table10). Nevertheless, difference in least squares mean for CON and NUT cows was detected (P=0.0923) for postpartum DMI contrast. Season of calving effects also were detected; cows that calved during the cooler months (SEA 2) had lesser DMI (P<0.0001) than cows that calved in SEA 1 through the postpartum period. A TRTxSEA interaction was detected (P<0.0001). Similar to the prepartum period, CON, NUT and MET groups of cows had greater DMI during SEA 2 compared to SEA 1. On the other hand, PPG cows had greater DMI during the cooler season (SEA 1). Differences in postpartum DMI (kg/d) between seasons (SEA 1 minus SEA 2) for the four treatment groups were 0.06, 3.6, 2.8, and -1.46 for CON, NUT, MET , and PPG. These differences between seasons differed ($P \leq 0.0234$) except for CON cows ($P=0.1734$). No significant orthogonal contrasts were detected for DMI ($P \geq 0.1598$) during this period (Appendix Table A).

No treatment effect was detected for BW during the early (+28 d) postpartum period ($P=0.1514$; Table 10). Nevertheless, orthogonal contrasts detected significant differences ($P=0.0525$) between CON and supplemented cows. Control cows (CON) weighed less (654.2 kg) compared to cows on NUT (661.3 kg), MET (670.3) and PPG (667.8) treatments. Season of calving did not affect BW during the postpartum period ($P=0.8081$). Least squares means for BW of cows during SEA 1 and SEA 2 were 662.7 and 663.6, respectively. No TRTxSEA interaction was detected ($P=0.8384$).

A treatment effect ($P<0.0673$; Table 10) for BCS was detected during the postpartum period. Differences in least squares means obtained from the mixed procedure of SAS showed that cows fed MET supplement had lower BCS during the postpartum period compared to NUT ($P=0.0195$) and PPG ($P=0.0280$) treatments (Figure 12). No season effect was detected for BCS during the postpartum period ($P=0.5140$; Figure 13). Least squares means were 3.2 for cows in the two seasons.

Overall evaluation of DMI, BW, and BCS results showed that PPG treated cows maintained BW and BCS slightly better with similar DMI after calving, whereas CON cows had numerically greater DMI postpartum and lost the greatest BW and BCS of all groups. Although cows fed CON and MET supplemented diets had similar BCS postpartum, BW during this period was greater for the cows in the MET group. This suggests that cows fed supplemented diets had improved energy balance compared to cows fed CON diet. However, other factors such as MY, concentrations and actions of various hormones also must be considered to better interpret meaning of the slight but often significant prepartum and postpartum changes in BW and BCS that were detected across treatments.

Overall, DMI during the transition period (-3 wk to + 4 wk) followed the expected pattern for cows at this stage and no effects of treatment on DMI were detected. Thus, there was no evidence that including glucogenic compounds in the transition diets caused greater or reduced DMI, although greater, but variable across treatments, intakes of precursors were consumed that could have been converted to glucose in the liver.

A normal lactation curve for Holstein cows shows an exponential increase in milk yield after calving that reaches a plateau (peak) around 5-7 wk into lactation after which milk production declines gradually throughout the remainder of the lactation. The level of production and the length of the lactation can be modified by many factors (e.g. nutrition and bST injections, among others). Some studies have shown a correlation between BCS at calving and milk yield. Broster and Broster (1998) reported 2 to 4 kg/d more milk for cows that calved with BCS of 3.25 compared to 2.0. A decrease in production also was seen when BCS was greater than 4.0 at calving. These effects on milk yield were only seen when differences in BCS that were compared were large. Compared to cows with low BCS at calving, cows with high BCS tended to produce milk with more fat at the beginning of lactation (Holter et al., 1990). This likely was due to a combination of reduced milk volume and the greater amount of mobilized body fat used directly for milk fat synthesis. On the other hand, milk protein did not appear to be affected by BCS at calving.

In the current experiment, milk production during first 150 d followed the pattern typically expected for Holstein cows. Means of milk production across treatments for the first 22 wk of lactation are in table 11. No significant effects of either calving BW or calving BCS were found on milk production. Similarly, there was no significant effect of treatment detected on 150 d milk production. However, during the 4 wk time period that glucogenic supplements were included in the TMR fed (wk 1 through 4), a significant treatment effect was detected. Overall, cows supplemented with PPG had lower milk yields than cows in the other three treatment groups (Figure 14). Also, during this 4 wk period, cows in CON group, which also had the greatest BCS at calving (3.7) produced slightly more milk (35.2 kg/d) than NUT (34.5 kg/d), MET (34.1 kg/d), or PPG (31.78 kg/d) supplemented cows which also had the lowest

Table 11. Least squares means and SE for milk production (kg/d) of Holstein cows during four periods through 22 wk of lactation when supplemented with glucogenic precursors during the transition period (-3 wk to +4 wk).

	Treatment[1]								Season[2]			
Period (wk)	CON	±SE[3]	NUT	±SE[3]	MET	±SE[3]	PPG	±SE[3]	SEA 1	±SE[3]	SEA 2	±SE[3]
1-4	35.2[b]	0.95	34.5[b]	0.89	34.1[b]	0.92	31.8[a]	0.92	35.1[b]	0.69	32.6[a]	0.62
5-10	42.2[b]	0.63	41.5[b]	0.59[b]	41.2[b]	0.61	40.3[a]	0.61	43.6[b]	0.45	38.9[a]	0.42
11-15	41.4	0.82	40.9	0.77	42.3	0.79	41.1	0.8	43.4[b]	0.59	39.4[a]	0.55
16-22	40.3	0.80	39.4	0.70	38.5	0.77	38.9	0.8	39.9	0.50	38.6	0.58

[1] CON= no supplement; NUT= 113 g/d of NutroCal; MET= 454 g/d of Metaxerol; PPG = 300 mL/d of propylene glycol.

[2] SEA 1= cooler (October-March); SEA 2=warmer (May-September).

[3] ±SE= plus or minus standard error.

[ab] Means with different letters within rows within treatment or within season differed ($P<0.05$; PDIFF).

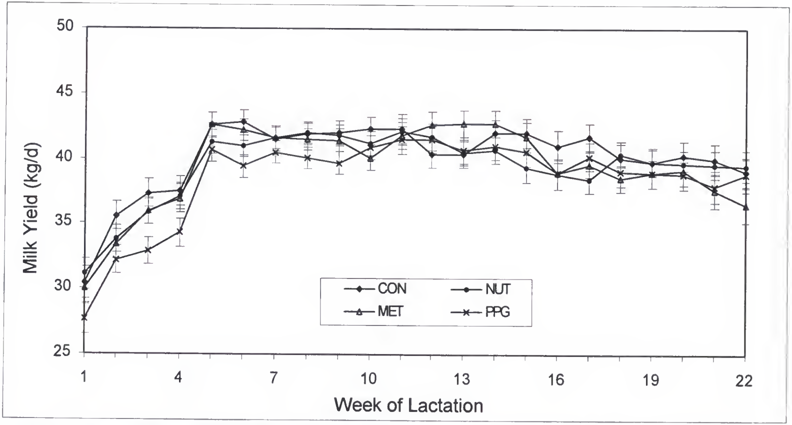

Figure 14. Least squares means and SE for mean weekly milk production through 22 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds. Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d propylene glycol.

calving BCS at calving (3.4). This agreed with the reported association between BCS and subsequent milk production described above.

Overall, no strong or significant associations were detected among DMI, BCS, and MY through the first 4 wk of lactation due to treatment . However, cows supplemented with NUT had similar mean DMI, BW and BCS prepartum as CON cows but tended to maintain greater BW and BCS postpartum while having similar postpartum DMI and they produced essentially the same amount of milk for 4 wk (-1.4%). Greater DMI during both prepartum and postpartum periods was seen for CON cows, but there was greater BW loss after calving, and similar mean 150-d milk yield compared to treated cows (39.8, 39.0, 39.0 and 38.0 kg/d for CON, NUT, MET and PPG, respectively). This suggests that cows supplemented with the glucogenic precursors better utilized nutrients and that feeding the glucogenic supplements for the time period tested in the current experiment may have had some beneficial effects.

As expected, cows that calved during the cooler season (SEA 1) had greater ($P<0.0001$) milk yield through 15 wk (105 d) of lactation compared to SEA 2 (Table 11; Figure 15). However, no differences in milk production among treatments were detected when production through 150 d was considered. This probably occurred because as lactation progressed cows also moved into different seasons and the seasonal effects would gradually have been reversed, especially with respect to effects of temperature and humidity on milk production. As mentioned before, cows on SEA 1 (cooler) had lower DMI but had greater milk yields, suggesting that this group was more

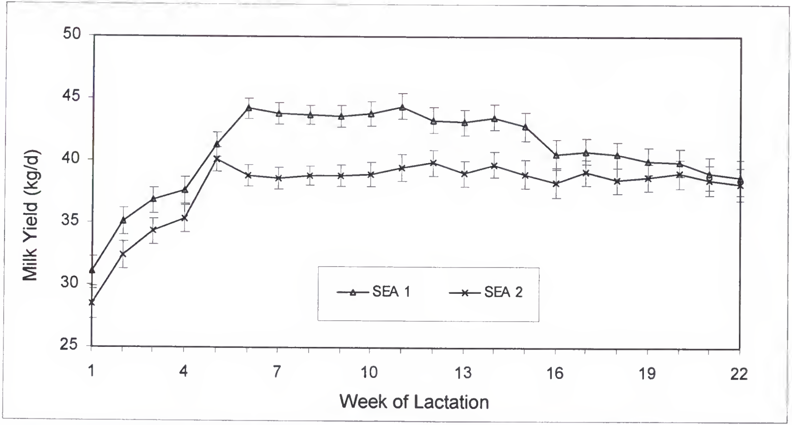

Figure 15. Least squares means and SE for mean weekly milk production from 3 wk prepartum through 22 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds during two seasons. Seasons were: SEA 1= cooler (October-March); SEA 2=warmer (May-September). Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d propylene glycol.

efficient. Although no direct measure was recorded such as temperature, humidity, or solar radiation, there was a season effect on DMI but this did not adversely affect milk production or BW or BCS of cows on the treatment diets.

Milk samples collected weekly were analyzed and least squares means for milk components measured (fat, protein and somatic cell count - SCC) through 70 DIM are in table 12. Mean percentage fat in milk and SCC were unaffected by glucogenic treatments, however, the effect of diet treatment on protein percentage in milk was significant ($P<0.001$). Cows on PPG had the greatest percentage of milk protein (2.88%) followed by MET (2.81%), CON (2.80%), and NUT (2.75%). This effect on milk protein likely was due to lower milk yield. Milk protein of CON cows was not significantly different from that of NUT or MET cows, however milk protein of PPG cows was greater compared to CON, NUT, or MET ($P\leq0.0204$; Table 12), and MET greater than NUT ($P=0.0837$). Calving season had no effect on SCC ($P=0.2494$). Milk fat was greater ($P=0.0010$) during SEA 1 (3.9%) compared to SEA 2 (3.7%). Milk protein was affected by calving season ($P=0.0010$); milk protein percentages were 2.79 and 2.83 for SEA 1 and SEA 2, respectively.

The energetic demands of cows by the end of gestation are 1.3 to 1.5 times greater than the maintenance requirements (Quigley and Drewry, 1998). During late pregnancy, energy requirements of Holstein cows are 2.3 Mcal/d (Bell, 1995). During lactation these requirements are increased to 26 Mcal/d of NE_L for cows producing 30 kg of milk/d. As mentioned previously, during both the prepartum and postpartum times of the transition period (-3 wk to + 4 wk),

Table 12. Least squares means and SE for milk component percentages and somatic cell count of Holstein cows through 70 DIM when supplemented with glucogenic precursors during the transition period (-3 wk to +4 wk).

	Fat (%)		Protein (%)		Somatic Cell Count (X10^3)	
Treatment^1	LSM^2	±SE^2	LSM^2	±SE^2	LSM^2	±SE^2
CON	3.8	0.6	2.80^{ab}	0.02	315	54
NUT	3.8	0.6	2.75^{a}	0.02	361	52
MET	3.8	0.6	2.81^{b}	0.02	393	53
PPG	3.7	0.6	2.88^{c}	0.02	431	54
Season^3						
SEA 1	3.9^{b}	0.5	2.79^{a}	0.01	344	39
SEA 2	3.7^{a}	0.4	2.83^{b}	0.01	406	37

[1] CON= no supplement; NUT= 113 g/d of NutroCal; MET= 454 g/d of Metaxerol; PPG = 300 mL/d of propylene glycol.

[2] LSM= Least squares means; ±SE= plus or minus standard error.

[3] SEA 1= cooler (October-March); SEA 2=warmer (May-September).

[abc] Means with different letters within columns within variables differed ($P<0.05$; PDIFF).

cows require more energy than they can consume, and this results in a negative energy balance with a concomitant loss of BW and BCS to meet their energy needs. Factors that affect the magnitude of the negative energy balance include BCS at calving, severity of DMI depression, quality of the ration, and season, among others. In many cases cows undergo great metabolic changes during the transition period that may adversely affect their performance and health status. Clearly, lactating cows depend greatly on glucose for energy to support milk production. Glucose is absolutely essential for mammary synthesis of milk and no other simple sugar can replace it. Correlation between milk yield and mammary uptake of glucose is about r=0.93. Transition cows have relatively high plasma concentrations of glucose and low plasma concentrations of NEFA. However, as they approach calving the available supply of glucose declines, whereas the demands increase (Figure 5) and this leads to a great imbalance in supply and demand for this important metabolite. Therefore, to meet their needs cows must increase synthesis of glucose via gluconeogenesis in the liver, and to a lesser extent in the kidney, from available precursors. This results in increased glucose availability but also increased mobilization of lipid from adipose and thus, increased concentrations of NEFA in plasma and fat accumulation in liver as triglycerides (Figure 6).

During the current study, plasma concentrations of both glucose and NEFA followed this aforementioned pattern in both CON cows and cows supplemented with glucogenic precursors. (Figures 5, 16 and 18). Glucose

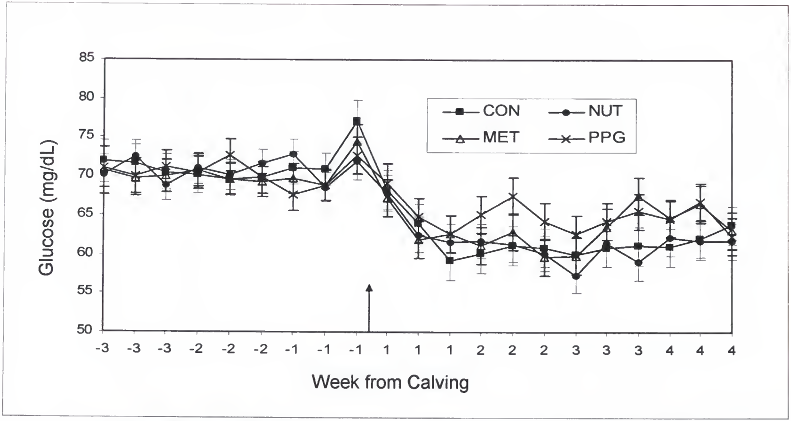

Figure 16. Least squares means and SE for glucose concentrations in plasma from 3 wk prepartum through 4 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds. Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Three samples taken per week. Arrow indicates calving.

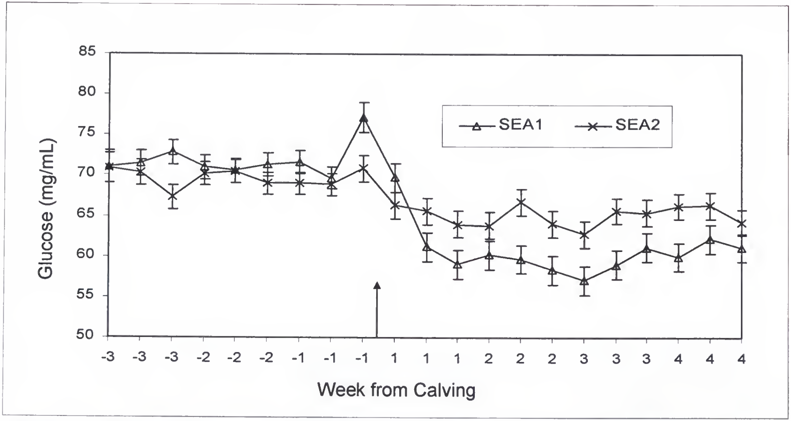

Figure 17. Least squares means and SE for glucose concentrations in plasma from 3 wk prepartum through 4 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds during two seasons. Seasons were: SEA 1= cooler (October-March); SEA 2=warmer (May-September). Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Three samples taken per week. Arrow indicates calving.

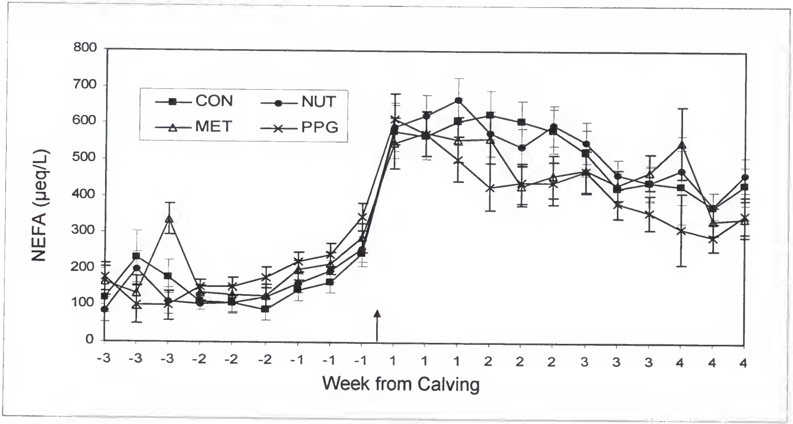

Figure 18. Least squares means and SE for NEFA concentrations in plasma from 3 wk prepartum through 4 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds. Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Three samples taken per week. Arrow indicates calving.

remained higher throughout the prepartum period until approximately 2 d prior to calving when a small peak was observed but then decreased significantly. Thereafter, concentrations of glucose remained lower throughout the postpartum period. During the prepartum period no differences in plasma glucose concentrations were detected due to treatment. However, cows that calved during SEA 1 tended to have greater (P= 0.0700) plasma glucose concentrations compared to cows that calved during SEA 2. Least squares means for plasma glucose concentrations are in table 13. A significant TRTxSEA interaction was detected (P=0.0084) for plasma glucose concentrations. Plasma glucose concentrations were similar among seasons for MET and PPG cows, whereas concentrations were greater for cows on SEA 1 compared to SEA 2 for CON (P=0.0207) and NUT (P=0.0897) cows. No orthogonal contrasts were detected for plasma glucose concentrations during the prepartum period.

Under most management conditions, where metabolic disturbances do not occur, plasma glucose levels begin to increase again at about 5 wk into the lactation. This increase is likely the result of increased DMI and a consequent reduction in the negative energy balance. In the current study, blood samples were only obtained during the 4 wk period that supplements were fed following calving so the longer term trends could not be evaluated. Plasma glucose concentrations decreased significantly after calving for approximately one week after which concentrations remained stable. Similar to the prepartum period, no differences were observed among treatments

Table 13. Least squares means and SE for plasma concentrations of glucose, NEFA and insulin of Holstein cows when supplemented with glucogenic precursors during the transition period (-3 wk to +4 wk).

	Glucose (mg/dL)				Insulin (ng/mL)				NEFA (μeq/L)			
Treatment[1]	PRE[2]	±SE[2]	POST[2]	±SE[2]	PRE[2]	±SE[2]	POST[2]	±SE[2]	PRE[2]	±SE[2]	POST[2]	±SE[2]
CON	71.4	1.3	61.8	1.8	0.72^{ab}	0.046	0.41	0.019	154.5^{ab}	16.2	515.7^{b}	28.2
NUT	70.9	1.1	61.5	1.6	0.77^{b}	0.041	0.41	0.017	149.6^{a}	13.1	529.4^{b}	25.7
MET	70.3	1.2	63.3	1.7	0.67^{a}	0.044	0.41	0.018	192.3^{c}	14.0	477.6^{ab}	26.9
PPG	70.5	1.2	65.0	1.7	0.76^{ab}	0.042	0.45	0.018	185.0^{bc}	14.0	430.3^{a}	26.9
Season[3]												
SEA 1	71.9^{b}	0.9	60.7^{a}	1.3	0.68^{a}	0.031	0.39^{a}	0.013	217.1^{b}	10.4	595.7^{b}	19.7
SEA 2	69.7^{a}	0.8	65.1^{b}	1.2	0.78^{b}	0.030	0.45^{b}	0.012	123.6^{a}	9.9	380.8^{a}	18.3

[1] CON= no supplement; NUT= 113 g/d of NutroCal; MET= 454 g/d of Metaxerol; PPG = 300 mL/d of propylene glycol.

[2] PRE= prepartum (-3 wk to calving); ±SE= plus or minus standard error; POST= postpartum (calving to +4 wk).

[3] SEA 1= cooler (October-March); SEA 2=warmer (May-September).

[abc] Means with different letters within columns within treatment or within season differed ($P<0.05$; PDIFF).

during the postpartum period. Contrary to what was found during the prepartum period, cows that calved during the cooler season (SEA 1) had lower mean plasma glucose concentration during the postpartum period (P=0.0126). No significant orthogonal contrasts or TRTxSEA interaction were detected during the postpartum period for plasma glucose concentrations.

Mean plasma NEFA concentrations differed among treatments prepartum (P=0.0760) and by season of calving (P<0.0001). Mean plasma NEFA concentrations are in table 13. Cows supplemented with NUT had lowest mean plasma NEFA concentrations (149.6 μeq/L) during the prepartum period compared to MET (192.3μeq/L; P=0.0275) and PPG (185.0 μeq/L; P=0.0663) treatment cows, and were slightly but not significantly lower than in CON cows (154.5 μeq/L; P=0.8125). Additionally, CON cows had lower plasma NEFA than cows supplemented with MET (P=0.0800) but not than PPG cows (P=0.1564). Cows fed MET and PPG had similar plasma NEFA concentrations (P=0.7128). Orthogonal contrasts corroborated differences between NUT and MET. However, concentrations in all cows, irrespective of supplementation, were less than that considered baseline (~ 200 μeq/L; Overton, 2000) during the 3 wk close-up period. Cows that calved during the cooler season (SEA 1) had greater (P<0.0001) plasma NEFA concentrations during the prepartum period. No significant SEAxTRT interaction was detected. At calving, mean concentrations of NEFA had increased approximately 300% (P<0.0001; Figure 18). Highest concentrations were found during the first week of the lactation, but thereafter they slowly declined as DMI increased and adipose tissue mobilization likely also declined. Differences among means for NEFA were detected after calving (P=0.0447). Control (CON) and NUT cows, which during the prepartum period had lower plasma NEFA concentrations, had greater plasma NEFA concentrations during the postpartum period than did PPG supplemented cows. Least squares means were 515.7, 529.4, 477.6, and 430.3 µeq/L for CON, NUT, MET, and PPG, respectively (Table13). Cows supplemented with PPG had lower plasma NEFA concentrations compared to CON (P=0.0303) and NUT (P=0.0088) treated cows, and numerically lower than MET supplemented cows (P=0.2165), but no other significant treatment differences were detected after calving.

A likely explanation for these results is that there were numerically, but not significantly, lower plasma concentrations of glucose in CON and NUT supplemented cows during the postpartum period. Nevertheless, orthogonal contrasts detected lower plasma NEFA concentrations for PPG compared to NUT and MET (P=0.0271). Similarly for seasons, cows that calved during SEA 1 had lower plasma glucose and greater plasma NEFA during the postpartum period (Figures 17 and 19). This pattern likely resulted because of the lower DMI for cows during SEA 1 during both prepartum and postpartum periods. A significant TRTxSEA interaction was detected (P=0.0610). Control (CON) cows that calved during SEA 2 had greater plasma NEFA concentrations (466 µeq/L) compared to MET (338 µeq/L) and PPG (330 µeq/L) supplemented cows ($P \leq 0.0191$), and numerically greater plasma NEFA concentrations

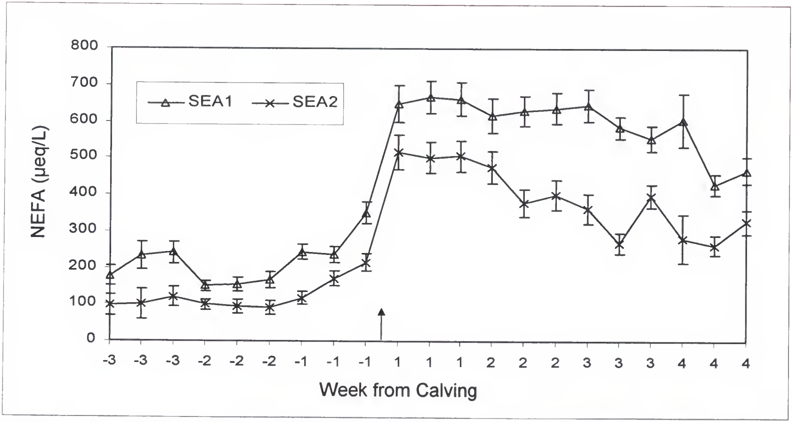

Figure 19. Least squares means and SE for NEFA concentrations from 3 wk prepartum through 4 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds during two seasons. Seasons were: SEA 1= cooler (October-March); SEA 2=warmer (May-September). Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Three samples taken per week. Arrow indicates calving.

compared to NUT supplemented cows (P=0.1319). During the cooler season (SEA 1) NUT supplemented cows had grater plasma NEFA concentrations than CON and PPG supplemented cows (672 vs. 564 and 529 µeq/L for NUT, CON and PPG, respectively; $P \leq 0.0551$). This suggests there was greater capacity of the glucogenic supplements to reduce plasma NEFA concentrations with greater dry matter intakes.

The amounts and proportions of VFA produced by fermentation of different diets are not constant, but rather are influenced by microbial fermentation balance, the escape of nutrients from the rumen and endogenous production by body tissues. Because most VFA are produced by rumen fermentation of the diet, the contribution of rumen VFA is lower as a proportion of net energy in a fasted animal than in one fed to satiety. The endogenous production of acetate causes net acetate utilization in the two dietary situations to be fairly similar, as opposed to the case of butyrate or propionate. Although butyrate is an important precursor of β-HBA in well-fed animals, in the fasting-state β-HBA is derived from endogenous fatty acids that are mobilized from adipose tissue as NEFA and partially metabolized to β-HBA (Van Soest, 1994). These two fatty acids must traverse through butyryl- CoA and/or acetyl-CoA and then condense with available oxaloacetate in order to enter the citric acid cycle. Fatty acids are utilized by liver at a specific rate. After entering cells, FA are converted to fatty acyl-CoA by acyl-CoA synthetase. Regulation of fatty acyl-CoA flow into mitochondria results from the fact that acyl-CoA derivatives cannot diffuse freely across the mitochondrial inner membrane. First they must to be converted to their acylcarnitine derivatives. This reaction is catalysed by the enzyme carnitine palmitoyltransferase 1 (CPT1) which spans the outer mitochondrial membrane, and it is considered the most critical step in controlling fatty acid flux through the β-oxidative pathway (McGarry and Brown 1997). During high energy demands (i.e. during early lactation) fatty acids are mobilized at a higher rate than they can be utilized resulting in partial utilization thus forming ketone bodies (including β-HBA) and TG infiltration and storage in the liver.

Clinical and sub-clinical ketosis results when plasma β-HBA concentrations increase above 14.4 mg/dL. Ketogenesis is regulated at three crucial steps: 1) Adipose tissue: factors regulating mobilization of free fatty acids from adipose tissue are important in controlling ketogenesis; 2) Liver: after acylation, fatty acids undergo ß-oxidation or are esterified to glycerol to form triacylglycerols or form ketone bodies. CPT-1 regulates entry of long-chain acyl groups into mitochondria prior to ß-oxidation. Its activity is low in the fed state, but is high during starvation. Malonyl-CoA formed in the fed state is a potent inhibitor of CPT-1. Under these conditions, free fatty acids enter the liver cell in low concentrations and are nearly all esterified to acylglycerols and transported out as VLDL. During starvation free fatty acid concentration increases, acetyl-CoA carboxylase is inhibited and malonyl-CoA decreases and this reduces the inhibition of CPT-I and allows greater ß-oxidation. These events are reinforced during starvation by a decrease in insulin/glucagon ratio. This causes inhibition of acetyl-CoA carboxylase in the liver by phosphorylation. In short, ß-oxidation from free fatty acids is controlled by the CPT-I gateway into the mitochondria, and the balance of free fatty acid uptake not oxidized is esterified. 3) Acetyl-CoA formed from ß-oxidation of fatty acids is either oxidized in TCA cycle or it forms ketone bodies.

It is now accepted that 14.4 mg of β-HBA /dL of plasma can be used as the cut-off point to discriminate between healthy cows and those with subclinical ketosis (Duffield et al., 1997, 1998b; Geishauser et al., 1998; Enjalbert et al., 2000). Sub-clinical ketosis most often occurs from 4 to 8 wk after calving. Feeding glucogenic supplements such as PPG (Burhans et al., 1997) and NutroCal (Tsang et al., 1997) reduces plasma β-HBA concentrations. Burhans et al. (1997) determined that twice-daily drenching of cows with propylene glycol (500 g/d) decreased concentrations of NEFA and ketone bodies in plasma of cows during the transition period. In a field study, Tsang et al. (1997) fed 114 g of NutroCal/d/cow for 3 wk prepartum and a minimum of 3 wk postpartum. This supplementation decreased NEFA concentrations during week 1 postpartum and decreased urine ketone score during wk 1 and 2 postpartum. Sorenson et al. (2001) supplemented 12 cows, of a greater group of 24 cows, with 450 g of Metaxerol/d/cow and reported increased DMI during the periparturient period. They suggested that the increased DMI and plasma glucose concentrations could be beneficial in preventing ketosis.

In the current study, plasma β-HBA concentrations followed expected patterns for lactating cows (Figures 20 and 21) that did not show clinical

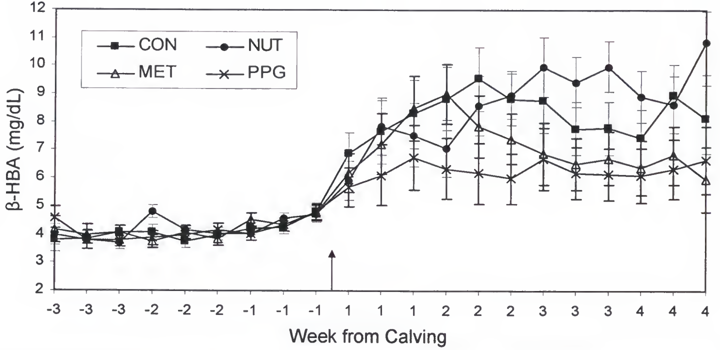

Figure 20. Least squares means and SE for β-hydroxybutyrate (β-HBA) concentrations in plasma from 3 wk prepartum through 4 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds. Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON :control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Three samples taken per week. Arrow indicates calving.

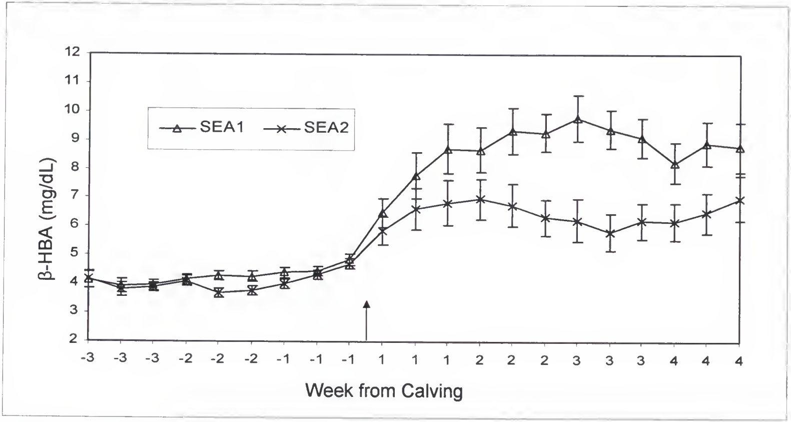

Figure 21. Least squares means and SE for β-hydroxybutyrate (β-HBA) concentrations in plasma from 3 wk prepartum through 4 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds during two seasons. Seasons were: SEA 1= cooler (October-March); SEA 2=warmer (May-September). Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d propylene glycol. Three samples taken per week. Arrow indicates calving.

metabolic problems during the transition period. Mean plasma β-HBA concentrations remained at basal levels (4.16 ±0.07 mg/dL) during the prepartum period but increased gradually during the last week prepartum as calving approached. After calving, plasma β-HBA concentrations increased and remained elevated thereafter. Least squares means for plasma β-HBA across treatments and SEA are in table 14. Plasma β-HBA concentrations were similar across treatments (P=0.9426) during the prepartum period (4.1, 4.2, 4.2, and 4.2 for CON, NUT, MET, and PPG, respectively) but tended to differ (P<0.0618) after calving (8.2, 8.6, 7.1, and 6.2 for CON, NUT, MET, and PPG, respectively), but still were less than concentrations that indicated metabolic problems. There was a tendency (P=0.0922) for cows that calved during the cooler season (SEA 1) to have greater plasma β-HBA concentrations (4.3 vs 4.0 mg/dL for SEA 1 and SEA 2, respectively) during the prepartum period.

Although mean difference in β-HBA concentration was small during the prepartum period, the difference in mean concentration differed (P=0.0016) during the 4 wk postpartum period (8.7 vs 6.4 mg/dL for SEA 1 and SEA 2, respectively). Although a TRTxSEA interaction was detected during the prepartum period (P=0.0622), plasma β-HBA concentrations between SEA were similar within each treatment except for NUT cows (P=0.0021), which had slightly greater β-HBA concentrations during SEA 1 (4.6 vs. 3.8 mg/dL). The TRTxSEA interaction differed (P=0.0258) after calving. All treatment groups had numerically greater plasma β-HBA concentrations during SEA 1

Table 14. Least squares means and SE for plasma IGF-I, calcium, and β-HBA of Holstein cows when supplemented with glucogenic precursors during the transition period (-3 wk to +4 wk).

	IGF-I (ng/mL)				Calcium (mg/dL)				β-HBA (mg/dL)			
Treatment[1]	PRE[2]	±SE[2]	POST[2]	±SE[2]	PRE[2]	±SE[2]	POST[2]	±SE[2]	PRE[2]	±SE[2]	POST[2]	±SE[2]
CON	259.1^{b}	9.0	139.9^{a}	7.3	8.39^{a}	0.14	8.58	0.15	4.09	0.14	8.24^{b}	0.73
NUT	256.3^{b}	8.1	144.6^{a}	6.7	8.68^{ab}	0.13	8.71	0.14	4.20	0.13	8.64^{b}	0.67
MET	229.8^{a}	4.5	142.7^{a}	7.0	8.74^{b}	0.13	8.73	0.14	4.18	0.13	7.12^{ab}	0.70
PPG	255.3^{b}	8.4	165.1^{b}	7.0	8.45^{ab}	0.13	8.53	0.14	4.16	0.13	6.24^{a}	0.70
Season[3]												
SEA 1	244.5	6.2	129.6^{a}	5.1	8.67	0.10	8.81^{b}	0.10	4.27	0.10	8.69^{b}	0.51
SEA 2	255.7	5.8	166.6^{b}	4.7	8.46	0.09	8.47^{a}	0.10	4.04	0.09	6.44^{a}	0.47

[1] CON= no supplement; NUT= 113 gr/d of NutroCal; MET= 454 gr/d of Metaxerol; PPG = 300 ml/d of propylene glycol.

[2] PRE= prepartum (-3 wk to calving); ±SE= plus or minus standard error; POST= postpartum (calving to +4 wk).

[3] SEA 1= cooler (October-March); SEA 2=warmer (May-September).

[ab] Means with different letters within columns within treatment or within season differed ($P<0.10$).

but differences were only significant for NUT (11.2 vs. 6.07; P=0.0002) and MET (8.9 vs. 5.4; P=0.0139). Differences between seasons for NUT and MET treated cows likely are the result of greater milk yields through 4 wk of lactation for these groups of cows, while the DMI was similar (NUT; 16.0 vs. 16.6 kg/d for SEA 1 and SEA 2, respectively) or lower (MET; 17.1 vs. 19.9 kg/d for SEA 1 and SEA 2, respectively). This resulted in lower glucose concentrations and likely required cows in these groups to rely to a greater degree on fat reserves for energy. Thus, NEFA and β-HBA concentrations in plasma increased. Orthogonal contrasts showed that plasma β-HBA concentrations during the postpartum period were lower for PPG compared to MET and NUT (P=0.00542) and differences in least squares means detected lower plasma β-HBA concentrations for PPG compared to CON (P=0.0487) and NUT (P=0.0139).

Mean plasma β-HBA concentrations across all treatments were less than the cut-off concentrations used to indicate subclinical ketosis (<14.4 mg/dL). Propylene glycol, which has been used extensively for treating ketosis, but usually by drenching and not supplemented in the feed, was more effective in the current experiment in preventing subclinical ketosis than NUT or MET based on plasma glucose, NEFA and β-HBA concentrations. This agrees with most reports that PPG reduces concentrations of plasma β-HBA (Studer et al., 1993; Grummer et al., 1994; Pickett et al., 2003).

Across treatments and seasons, plasma β-HBA concentrations increased and remained at higher levels after calving. Greater plasma β-HBA concentrations after calving suggested greater energy demands due to milk production, thus increased mobilization of fatty acids to cope with increased energy requirements. Lower plasma β-HBA concentrations in cows supplemented with PPG could be due to: 1) lesser milk yield of this group of cows with resultant reduced energy requirements and reduced requirement to mobilize fatty acids from adipose; 2) the moles of propionate produced from PPG may have been greater than from NUT or MET. Indeed this seems possible since greater quantity of PPG (300 g/d) was supplemented compared to 114 g/d of NutroCal and probably also greater to the glucogenic potential of the 0.450 kg of Metaxerol fed per day; 3) PPG may increase the availability of oxaloacetate required to condense with fatty acids so they can enter into the citric acid cycle; and finally, 4) PPG may regulate fatty acid mobilization and/or rate of utilization at the molecular level.

Similar results for NEFA and β-HBA in response to supplemental PPG were reported by Burhans et al. (1997). They determined that drenching cows twice daily with PPG (500 g/d) decreased concentrations of NEFA and β-HBA in plasma of cows during the transition period. Studer et al. (1993) drenched multiparous cows with 1 L of PPG once daily beginning 10 ± 3.6 d prior to calving until calving. This group reported reduced plasma β-HBA concentrations prepartum with no effects on prepartum or postpartum DMI or milk yield and composition. However, they reported no effects on plasma β-HBA concentrations after calving, when PPG supplementation ceased. Nevertheless, concentrations of plasma β-HBA during both prepartum and postpartum were greater than those reported by most researchers; probably due to different laboratory analysis techniques, diet composition or quantities of nutrients fed, which agreed with Grummer et al. (1994). As they increased the PPG dose, the acetate to propionate ratio in rumen declined in addition to lower β-HBA, suggesting, as the authors mentioned, some conversion to propionate. Pickett et al. (2003) drenched Holstein cows with 500 mL/d of PPG during the first 3 wk postpartum. They reported a tendency (P=0.1100) for plasma β-HBA concentrations to decrease during the first week postpartum, but β-HBA concentrations were not significantly affected by treatment from d 2 through 21 postpartum. Christensen et al. (1997) and Stokes and Goff (2001) reported that PPG administration did not affect plasma β-HBA concentrations. Differences between these reports, including the current study, suggests that PPG has a short-term effect and although it does not increase milk yield if supplemented during the periparturient period, it may reduce the incidence of ketosis (measured indirectly in the current study by plasma β-HBA concentrations).

Limited designed research has been published on the use of NUT and MET and their effects on plasma concentrations of β-HBA. In the current study, NUT cows had greater plasma concentrations of β-HBA during the postpartum period compared to PPG (P=0.0139) and tended to be greater compared to MET (P=0.1166), but not compared to CON (P=0.6863), as previously mentioned. Beem et al. (2003) reported no significant effects on β-HBA when they fed cows 113.5 g/d of NUT prepartum. NutroCal cows had numerically greater plasma β-HBA concentrations (7.9 vs. 8.3 mg/dL) when fed diets at 105% of energy requirements (NRC, 2001) and numerically lower concentrations when fed 145% of energy requirements (9.6 vs 7.2 mg/dL). Sorenson (unpublished; personal communication from R. Patton) fed 24 multiparous cows 454 g of Metaxerol/d starting approximately 3 wk prior to calving and found no significant differences on plasma β-HBA concentrations between treated and untreated cows during the treatment period.

Overall, cows fed PPG had reduced plasma β-HBA concentrations compared to CON and NUT cows, thus potentially reducing the chances of ketosis occurring. Metaxerol, which contains PPG and propionate, among other constituents, showed a tendency to reduce plasma β-HBA concentrations during the postpartum period. Least squares mean concentrations for the postpartum period were 8.24, 8.64, 7.12 and 6.24 mg/dL for CON, NUT, MET, and PPG cows (Table 14).

Approximately one-half of multiparous dairy cows experience moderate to severe fatty liver at calving (Grummer, 1995). It has been shown that fatty liver may increase the incidence of mastitis, displaced abomasum (DA), retained placenta (RP) and metritis, in addition to poor reproductive performance (Gerlof et al., 1986) and immune suppression (Reid and Williams, 1984). Cows with fatty liver are more prone to develop ketosis which may result in decreased milk production. As mentioned above, greater amounts of fatty acids from adipose tissue are mobilized during the transition period and early lactation due to increased energy demands and insufficient DMI. Fatty liver occurs when the rate of hepatic synthesis of triglycerides exceeds the rate of triglyceride removal through hydrolysis or via secretion of VLDL. Microsomal triglyceride transfer protein (MTP) transfers fatty acids from outside the endoplasmic reticulum into the lumen and therefore, it plays a critical role in coordinating the assembly of VLDL constituents. Microsomal triglyceride transfer protein determines the rate of VLDL secretion and MTP's concentration and/or activity are unaffected by increasing concentrations of NEFA, which are observed during the transition period (Bremmer et al., 2000). Reports suggest that accumulation of lipids in liver during negative energy balance may decrease the ability of the liver to mobilize triglycerides as VLDL (Reid et al., 1979; Pullen et al., 1988). This indicates that fatty liver is largely a function of nutritional status. The rate of gluconeogenesis in fatty liver is not optimal which results in prolongation of increased lipolysis, particularly during the first week after parturition (Rukkwamsuk et al., 1999).

Livers are considered to be fatty when the percentage of total fat in the liver equals or exceeds 14% of wet liver weight. Overall, least squares means for liver fat on a wet weight basis in the current study were 3.6% prepartum and 7.9% after calving. On average these were well below the accepted percentages that describe fatty liver. Furthermore, no great differences in liver infiltration with fat were seen during current study. Least squares means for total liver fat are in table 15. No treatment (P=0.9672) or season (P=0.3407) effects on percentage liver fat were detected during the prepartum period and

Table 15. Least squares means and SE for percentage total liver fat of Holstein cows when supplemented with glucogenic precursors during the transition period (-3 wk to + 4 wk).

	Percentage liver fat[1]					
Treatment[2]	Prepartum[3]	±SE[3]	Postpartum[3]	±SE[3]	Overall	±SE[3]
CON	3.27	1.00	7.69	1.53	6.53	1.35
NUT	3.99	1.12	10.04	1.66	8.59	1.47
MET	3.49	1.09	6.31	1.67	6.33	1.47
PPG	3.74	1.12	7.75	1.62	6.46	1.44
Season[4]						
SEA 1	4.15	0.65	8.44	0.91	7.33	0.82
SEA 2	3.10	0.87	7.45	1.34	6.62	1.18

[1] As percentage of total liver in wet weight basis.
[2] Treatments were: CON= no supplement; NUT= 113 g/d of NutroCal; MET= 454 g/d of Metaxerol; PPG = 300 mL/d of propylene glycol.
[3] Prepartum= -3 wk to calving; ±SE= plus or minus standard error; postpartum= calving to +4 wk.
[4] Seasons were: SEA 1= cooler (October-March); SEA 2=warmer (May-September).

means were similar. Similarly, no treatment (P=0.4705) or season (P=0.5431) effects were detected postpartum. Although differences among treatments did not differ statistically, liver of NUT cows did have ≈30% more fat compared to CON and PPG, and 58% more fat than MET supplemented cows. Overall, mean total fat percentages in liver were 6.5, 8.6, 6.3 and 6.4 % for CON, NUT, MET, and PPG, and 7.3 and 6.6 % for cows that calved during SEA 1 and SEA 2. Analyses showed a sample (P<0.0001) and treatment by sample effect (P=0.0021), where lowest fat percentages were found on d -21. Percentage liver lipid increased after calving with greatest percentages on d +14, but at +28 d postpartum percentages were similar to percentages found around calving (Figures 22 and 23). Total liver fat percentage across treatments for the four sample periods were 3.9, 6.6, 9.9, and 7.6 for -21 d, around calving, +14 d and +28 d, respectively. These percentages differed from one another (P<0.0001) except for the comparison of samples taken around calving and at +28 d (P=0.1162). This increase of fat infiltration into the liver after calving was expected due to greater fatty acid mobilization, increased energy demand, and lagging increase in DMI. Greater liver fat percentage for NUT cows did correspond with lower plasma concentrations glucose and greater plasma concentrations of NEFA and β-HBA for this group during the postpartum period. This suggests that cows supplemented with NUT likely had to mobilize energy reserves from adipose to a greater extent to cope with energy demand postpartum resulting in greater NEFA in circulation. Thus, uptake of fatty acids

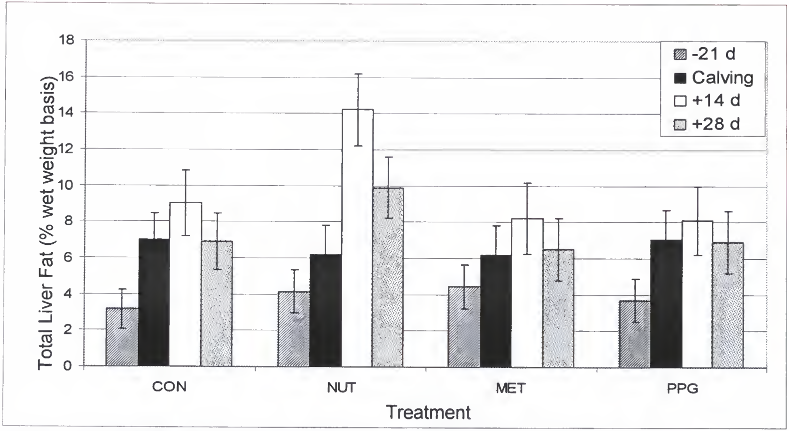

Figure 22. Percentage of total liver fat of Holstein cows fed diets supplemented with glucogenic compounds during the transition period (-3wk to + 4 wk). Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol.

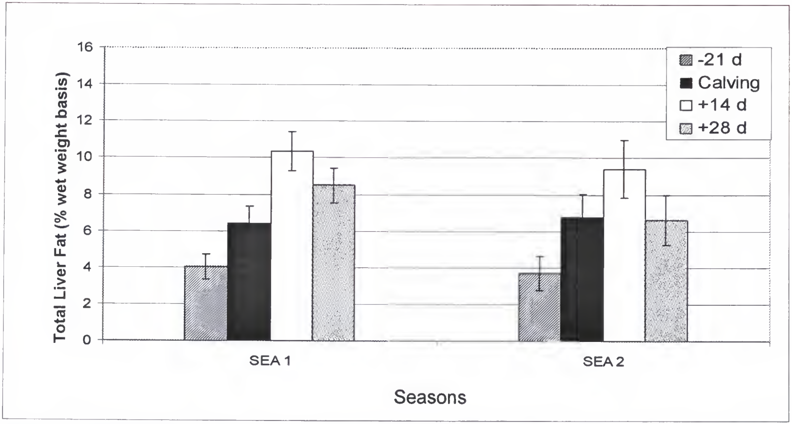

Figure 23. Percentage of total liver fat of Holstein during two different calving seasons. Seasons were: SEA 1= cooler (October-March); SEA 2=warmer (May-September).

by the liver resulted in some additional TG stored in liver and also increased ketone body production and β-HBA concentration in plasma.

Insulin concentrations of cows are highly responsive to plane of nutrition. Feed restriction is associated with depressed serum insulin concentrations in lactating cattle. Serum insulin concentrations are reduced to a greater extent in high yielding cows that are underfed. Increased hepatic glucose output is promoted by decreased circulating insulin in ruminants, even though the number of insulin receptors of hepatocytes does not change during lactation. Insulin concentrations are depressed during lactation which enhances plasma glucose concentrations and its availability to the high demands of the mammary gland.

In the current study, overall mean plasma insulin concentration on prepartum d -21 was 0.92 ng/mL and gradually declined as calving approached; it was 0.43 ng/mL after calving and then remained low during the postpartum period (Figures 24 and 25). These changes in plasma insulin concentrations were likely due to the lagging increase in DMI and increased demands for glucose and other metabolites for milk production. Least squares means for plasma insulin during the prepartum period were 0.72, 0.77, 0.67, and 0.76 ng/mL for CON, NUT, MET, and PPG, and they did not differ across treatments (Table 13). Nevertheless, orthogonal contrasts and differences of least squares means did indicate greater (P=0.0768) plasma insulin concentrations for NUT supplemented cows compared to those supplemented with MET. Cows that calved during the cooler season (SEA 1) had lower

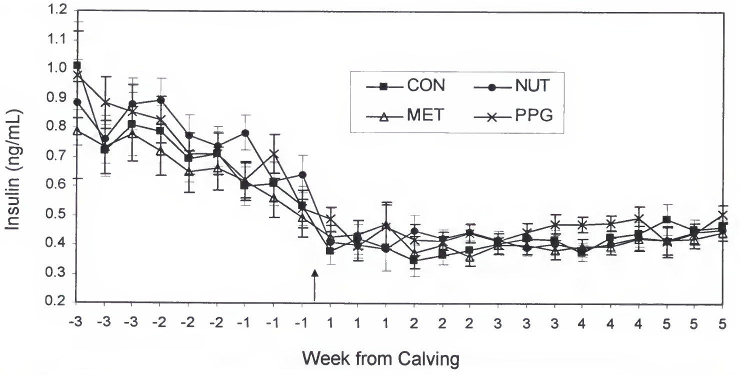

Figure 24. Least squares means and SE for insulin concentrations in plasma from 3 wk prepartum through 5 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds. Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Three samples taken per week. Arrow indicates calving.

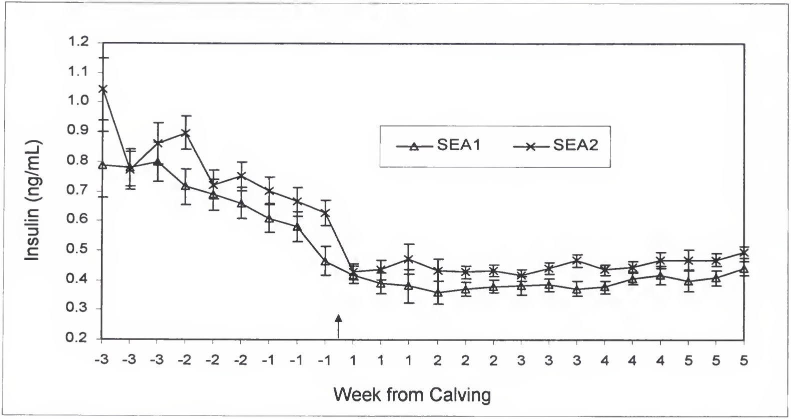

Figure 25. Least squares means and SE for insulin concentrations from 3 wk prepartum through 5 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds during two seasons. Seasons were: SEA 1= cooler (October-March); SEA 2=warmer (May-September). Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Three samples taken per week. Arrow indicates calving.

plasma insulin concentrations during the prepartum period compared to cows that calved during SEA 2 (0.68 vs. 0.78 ng/mL). No interaction between season of calving and dietary supplement was detected ($P=0.5807$).

Similarly to the findings for the prepartum period, no treatment effect was detected during the postpartum period, but cows that calved during SEA 1 had lower mean plasma insulin concentrations than cows calving during SEA 2 (0.39 vs. 0.45 ng/mL; $P=0.0023$). There was a tendency ($P \geq 0.0945$) during the postpartum period for PPG supplemented cows to have greater plasma insulin concentrations compared to NUT and MET groups. Least squares means for plasma insulin concentrations during the postpartum period were 0.41, 0.41, 0.41, and 0.45 ng/mL for CON, NUT, MET, and PPG. No TRTxSEA interaction on plasma insulin was detected after calving. Cows calving during SEA 1 had lower plasma insulin compared to SEA 2 cows probably due to lower DMI by cows during this season. The lack of insulin response to supplemental glucogenic compounds is consistent with reports from studies conducted with beef (Waterman et al., 2002; Endecott et al., 2003) and dairy cows (Akinyode et al., 2001; Pickett et al., 2003). On the other hand, Grummer et al. (1997) reported a linear increase in insulin concentrations of Holstein heifers when restricted to 50% of *ad libitum* intake and drenched for four consecutive days before calving with either 0, 296, 592 or 887 mL of PPG. Differences among studies likely were due to different quantities of PPG used and blood sampling schemes.

Dietary protein and energy intake, and insulin, among other factors, have been found to regulate synthesis, secretion, and circulating concentrations of IGF-I. This growth factor has been found to increase mammary cell numbers and increase milk secretion when infused into the blood supply of ruminants (McGuire et al., 1992). The liver, which is the major source of IGF-I, shows reduced IGF-I synthesis during early lactation and this agreed with lower IGF-I concentrations prepartum and during early postpartum periods. Low IGF-I is associated with the negative energy balance during early lactation (Breier et al., 1986; Spicer et al., 1990; McGuire et al., 1992). As expected, plasma concentrations of IGF-I were reduced around calving and increased gradually thereafter (Figures 26 and 27). Mean prepartum concentrations of IGF-I (Table 14) were affected by treatment ($P=0.0574$) but not by season. Cows supplemented with MET had lower (11-12%) plasma IGF-I concentrations (229.8 ng/mL; $P\leq0.0343$) compared to CON (259.1 ng/mL), NUT (256.3 ng/mL), or PPG (255.3 ng/mL) supplemented cows. The orthogonal contrast for NUT and MET ($P=0.0253$) were significant. No significant SEA or TRTxSEA interaction was detected.

During the postpartum period treatment ($P=0.0485$) and season of calving ($P<0.0001$), but not their interaction, had significant effects on plasma IGF-I concentrations. After calving, PPG supplemented cows had greater plasma concentrations of IGF-I compared to CON, NUT, and MET groups, but this did not result in greater milk production by these cows. Greater plasma concentrations of IGF-I were detected for cows that calved during SEA 2. The

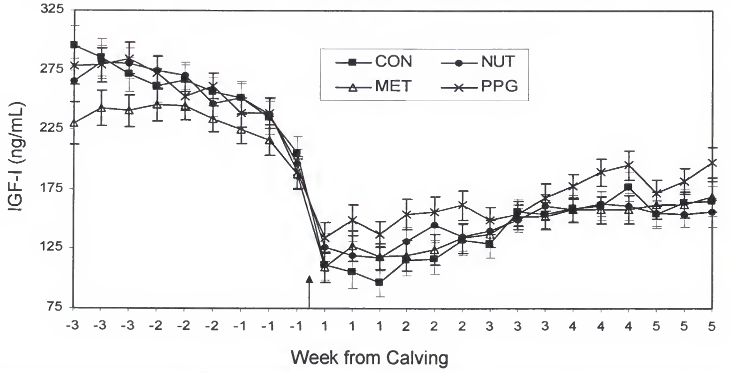

Figure 26. Least squares means and SE for IGF-I concentrations in plasma from 3 wk prepartum through 5 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds. Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Three samples taken per week. Arrow indicates calving.

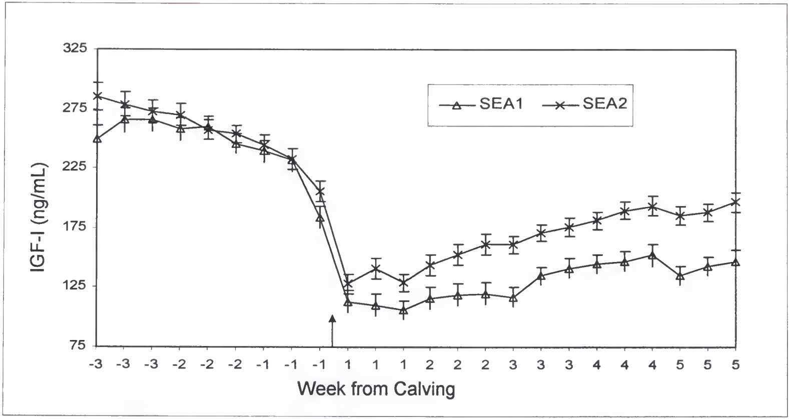

Figure 27. Least squares means and SE for IGF-I concentrations in plasma from 3 wk prepartum through 5 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds during two seasons. Seasons were: SEA 1= cooler (October-March); SEA 2=warmer (May-September). Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Three samples taken per week. Arrow indicates calving.

greater mean IGF-I concentration found during the postpartum period for PPG and also during SEA 2 likely were due to greater insulin and glucose concentrations, and the slightly greater DMI in these group of cows. This suggests even though IGF-I concentrations were highly correlated with milk yield, other factors also are involved that result in improved milk production (e.g., cows must reduce the negative energy balance).

The onset of lactation places a sudden large demand for Ca from the blood of dairy cows. In some cases, mammary drain of Ca causes blood Ca concentrations to drop drastically (from 9 - 10 mg/dl to <5mg/dl) resulting in hypocalcemia (milk fever). Although this decrease is relatively easy to treat, there is evidence (Goff and Horst, 1997) that cows that have milk fever or low blood Ca levels are more susceptible to other disorders such as mastitis, displaced abomasum, retained placenta, and ketosis. In the present study plasma Ca concentrations during the prepartum period were unaffected by either treatment or season of calving and no TRTxSEA interaction was detected. Least squares means for plasma Ca concentrations are in table 14.

No significant treatment effect or TRTxSEA interaction were detected during the postpartum period, but cows that calved during the cooler season (SEA 1) did have greater mean plasma Ca concentration during the postpartum period than cows that calved during SEA 2 (8.81 vs. 8.47 mg/dL; P=0.0189). Mean plasma Ca concentrations for both periods, prepartum and postpartum, remained above 8.4 mg/dL, except around calving when concentrations dropped but on average still were above 7 mg/dL. Despite overall means for the four treatment groups being over 7 mg/dL, many cows had plasma Ca concentrations below 7 mg/dL at any given time between one wk prepartum and one wk postpartum (incidences of plasma Ca concentrations below 7mg/dL: CON n=12; NUT n=13; MET n=12 and PPG n=17). After calving plasma Ca concentrations returned to concentrations seen prepartum (Figures 28 and 29). During the first week after calving a significant increase in plasma Ca concentration to ~10.7 mg/dL was observed for NUT treated cows, whereas CON, MET and PPG groups increased but only up to concentrations seen before calving (~ 8.9 mg/dL). This sudden increase in plasma Ca concentration on NUT treated cows may have been due to the supplementation of Ca in the form of calcium propionate. The amount of NUT supplemented (114 g/d/cow) in the current experiment supplied an additional 24.1 g of Ca each day.

Associations between DMI, BW, and BCS showed that treatments had no effect on DMI although NUT treated cows had slightly lower intakes after calving. The fact that DMI was only recorded up to 4 wk into lactation must be taken into consideration. Similar DMI resulted in similar BW across treatments throughout the duration of the current experiment (-21 d to + 150 d). Nevertheless, CON cows tended to lose more body condition during the postpartum period. All treatment groups maintained or increased BCS after 4 wk postpartum when dietary treatments stopped. During the first 4 wk after calving, PPG cows produced on average 8.8% less milk compared to CON, NUT, and MET supplemented cows. This may be one reason for better BW

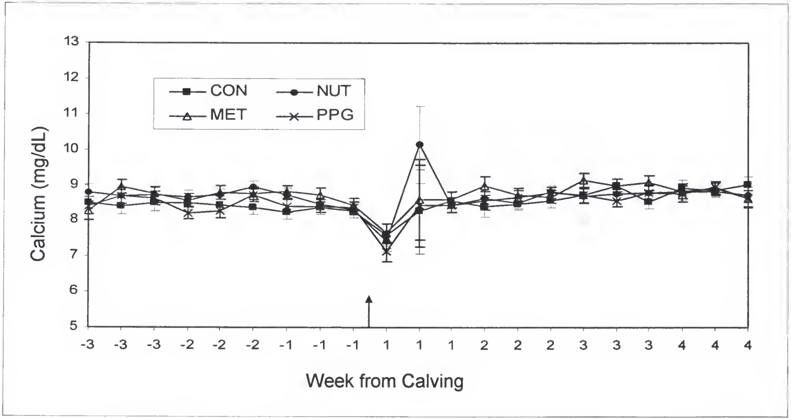

Figure 28. Least squares means and SE for calcium concentrations in plasma from 3 wk prepartum through 4 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds. Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Three samples taken per week. Arrow indicates calving.

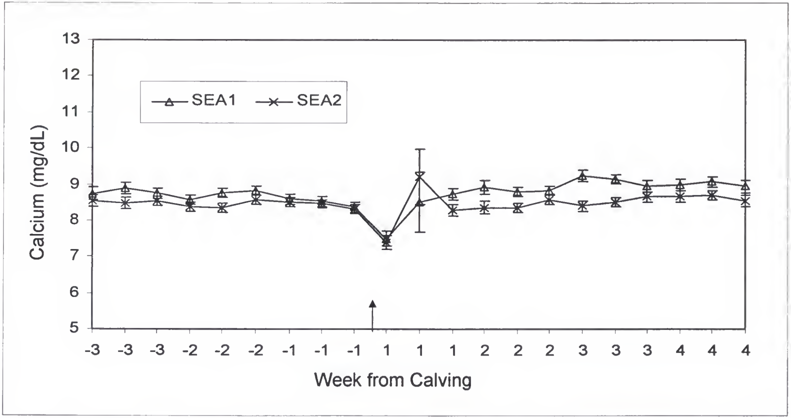

Figure 29. Least squares means and SE for calcium concentrations from 3 wk prepartum through 4 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds during two seasons. Seasons were: SEA 1= cooler (October-March); SEA 2=warmer (May-September). Treatments were from 3 wk prior to calving through 4 wk postpartum. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Three samples taken per week. Arrow indicates calving.

and BCS observed for this group of cows during the first 4 wk of lactation. On the other hand, differences between calving BW and BCS and overall postpartum BW and BCS of CON cows were greater compared to treated cows.

Overall, 150 d milk production did not differ across treatments. Contrary to what was expected, cows that calved during the cooler season (SEA 1) had lower DMI compared to cows calving during the warmer season during both the prepartum and postpartum periods. However, this did not result in lower BW, BCS, or milk yield. Milk yield during the first 4 wk of lactation, the time period when postpartum DMI was recorded, was on average 7.1% greater for cows that calved during the cooler season, whereas BW and BCS were unaffected by season of calving. This suggests that cows that calved during the cooler season were more efficient in utilization of nutrients. Dry matter intake was not improved by supplementing glucogenic precursors during either season.

Residual correlations for plasma hormones, growth factor, and metabolites analyzed in the current study were calculated. Correlations were for the prepartum period or for during the postpartum period (Table 16) and also for the overall transition period (-3 wk to + 4 wk; Table 17). Model used was: Y= TRT SEA TRT*SEA COW(TRT*SEA) DAY DAY^2 DAY^3. Some correlations detected during the prepartum period were unexpected. It was interesting that glucose was positively correlated with NEFA (r=0.077; P=0.026) during the prepartum period. This likely indicates that cows were in

Table 16. Residual MANOVA correlations for blood hormone, growth factor, and metabolites during prepartum and postpartum periods of Holstein cows supplemented with glucogenic precursors during the transition period (-3 wk to + 4 wk)[1].

Postpartum[2] Prepartum[2]	GLUCOSE	NEFA	INSULIN	CALCIUM	IGF-I	β-HBA
GLUCOSE		-0.095 P< 0.001	0.144 P< 0.001	-0.003 P= 0.894	0.108 P< 0.001	-0.217 P< 0.001
NEFA	0.077 P= 0.025		-0.141 P< 0.001	-0.002 P= 0.915	-0.193 P< 0.001	0.128 P< 0.001
INSULIN	0.003 P= 0.911	-0.130 P< 0.001		-0.037 P= 0.237	0.158 P< 0.001	-0.099 P< 0.001
CALCIUM	0.020 P= 0.564	-0.139 P< 0.001	0.081 P= 0.053		-0.009 P= 0.731	0.0001 P= 0.996
IGF-I	-0.035 P= 0.307	-0.326 P< 0.001	0.198 P< 0.001	0.157 P< 0.001		-0.166 P< 0.001
BHBA	-0.002 P= 0.953	0.213 P< 0.001	0.129 P< 0.001	-0.013 P= 0.702	-0.044 P= 0.213	

[1] Treatments were: CON= no supplement; NUT= 113 g/d of NutroCal; MET= 454 g/d of Metaxerol; PPG = 300 mL/d of propylene glycol. Model used was: y= trt sea trt*sea cow(trt*sea) day day^2 day^3.

[2] Prepartum values are below the diagonal line; postpartum values are above the diagonal line.

Table 17. Overall residual correlations (MANOVA) for blood hormone, growth factor and metabolites of Holstein cows supplemented with glucogenic precursors during the transition period (-3 wk to + 4 wk) [1].

	GLUCOSE	NEFA	INSULIN	CALCIUM	IGF-I	β-HBA
GLUCOSE		-0.156 P< 0.001	0.099 P< 0.001	0.001 P= 0.837	0.164 P< 0.001	-0.255 P< 0.001
NEFA	-0.155 P< 0.001		-0.141 P< 0.001	-0.009 P= 0.675	-0.304 P< 0.001	0.276 P< 0.001
INSULIN	0.098 P< 0.001	-0.141 P< 0.001		-0.001 P= 0.965	0.201 P< 0.001	-0.040 P= 0.061
CALCIUM	0.001 P= 0.837	-0.009 P= 0.675	-0.001 P= 0.965		0.027 P= 0.195	-0.001 P= 0.991
IGF-I	0.163 P< 0.001	-0.304 P< 0.001	0.201 P< 0.001	0.027 P= 0.195		-0.218 P< 0.001
BHBA	-0.255 P< 0.001	0.276 P< 0.001	-0.040 P= 0.061	-0.001 P= 0.991	-0.218 P< 0.001	

[1] Treatments were: CON= no supplement; NUT= 113 g/d of NutroCal; MET= 454 g/d of Metaxerol; PPG = 300 mL/d of propylene glycol. Model used was: y= trt sea trt*sea cow(trt*sea) day day^2 day^3

positive energy balance because of high level of DMI. However, correlation was negative (r=-0.095; P<0.001), as expected, after calving and for the overall transition period (-3 wk to + 4 wk; r=-0.156; P<0.001; Table 17).

Glucose and insulin were significantly correlated (r=0.099; P<0.001; Table 17) and correlations followed similar trends throughout the overall transition period (-3 wk to +4 wk; Figure 30). However, glucose and insulin were not correlated during the prepartum period (r=0.004; P=0.911; Table 16). This likely was due to a sudden increase in glucose observed before calving. Contrary to what was expected, prepartum β-HBA and insulin were positively correlation (r=0.130; P<0.001). However, they were negatively correlated during the postpartum period (r=-0.099; P<0.001) and for the overall transition period (r= -0.040; P=0.061). All variables, except for calcium, were significantly correlated during the postpartum period and during the overall transition period (-3 wk to +4 wk). Glucose concentrations decreased gradually as calving approached with a sudden increase prior to calving after which concentrations dropped. This drop most likely was due to greater demands by mammary gland to support milk synthesis. Insulin tended to follow the drop in glucose. Increased energy demands after calving required fatty acid mobilization from adipose tissue in an effort to cope with the negative energy balance. This resulted in increased concentrations of NEFA, which started to decline after 2 wk of lactation. However, fatty acid utilization by the liver was limited and excess fatty acids in liver likely were partially metabolized to β-HBA or stored in liver as triglycerides. Total liver lipids increased after

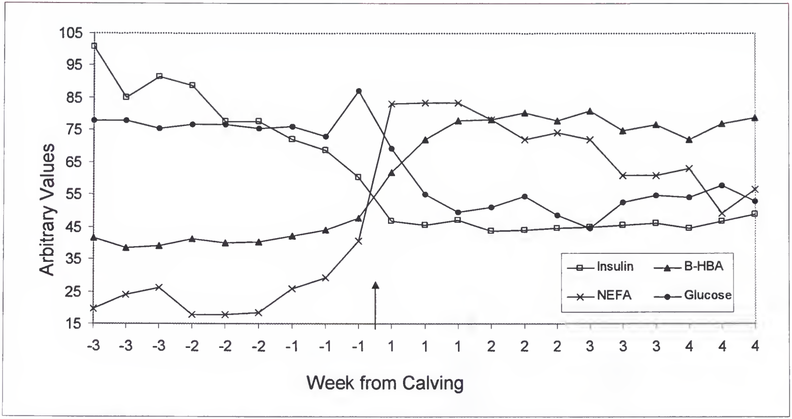

Figure 30. Trends of plasma insulin, β-HBA, glucose, and NEFA from 3 wk prepartum through 4 wk of lactation of Holstein cows fed diets supplemented with glucogenic compounds. Diets were: CON: control, no supplement; NUT: 114 g/d of NutroCal; MET: 454 g/d of Metaxerol; PPG: 300 mL/d of propylene glycol. Three samples taken per week.
Arrow indicates calving.

calving, with greater percentages seen 2 wk postpartum after which they returned to calving levels by 28 d of lactation. Plasma β-HBA concentrations after calving were highly correlated to NEFA ($P<0.001$), both indicating increased dependence of cows on utilization of mobilized fatty acids to meet early needs.

No significant differences were detected during the prepartum period for blood measures except for NEFA and IGF-I. Cows supplemented with MET had greater NEFA concentrations, and although not significant, they also had numerically lower concentrations of glucose and insulin (Table 13). This may explain, in part, lower IGF-I concentrations found for MET supplemented cows during this period. After calving, numerically lower DMI of NUT cows was reflected by numerically lower glucose and insulin concentrations, and greater NEFA and β-HBA concentrations. Moreover, total liver lipid was numerically greater for cows of this group.

Lower DMI of cows that calved during SEA 1 did not result in reduced plasma glucose concentrations during the prepartum period. Unexpectedly, glucose concentrations were greater for cows that calved during SEA 1. However, lower DMI during the prepartum period for this group of cows resulted in lower insulin concentrations, numerically lower IGF-I, greater NEFA, and numerically greater β-HBA. After calving, cows that calved during the cooler season (SEA 1) had lower DMI ($P<0.0001$) but greater milk yield. This situation resulted in a greater energy demand for these cows, likely reasons for lower glucose and insulin ($P\leq0.0126$) and greater NEFA and β-

HBA ($P \leq 0.0016$). Lower IGF-I concentrations for this group of cows (SEA 1) likely was due to a negative energy balance and low insulin concentrations. Nevertheless, no differences were found on liver total lipid percentages due to season of calving.

The number of cows used in the current experiment was not sufficient to draw conclusions on the effects of supplementing glucogenic precursors during transition on the incidence of health problems. However, no cases of ketosis were found and the incidence of health problems was similar across treatments. Moreover, incidences of health problems were within the expected range for the current herd. This was true except for MET where retained fetal membrane incidence (16.0%) was greater than the 10% expected. Additionally, metritis incidence for this group was greater (35.5%) compared to CON (17.2%), NUT (15.2%) and PPG (19.4%) groups, but no linked reason for this was obvious except for a greater incidence of retained fetal membranes. The incidence of displaced abomasum cases for CON (8.3%) and NUT (5.7%) was greater that the 5% expected. Greatest percentage of cows removed from the experiment was for the CON group (25.6%, 15.4%, 18.4%, and 13.9% for CON, NUT, MET, and PPG groups, respectively). However, it was concluded that supplementing cows at the current rates during the transition period caused no obvious harm nor benefit with respect to health.

Table 18. Number of incidences of health problems and distribution of cows removed from trial when fed glucogenic supplements during the transition period (-3 wk to +4 wk)[1].

	Incidences of Health Problems[2]					
Treatment	Mastitis	Retained Fetal Membranes	Milk Fever	Metritis	Other	Total
CON	3	3	1	5	2	14
NUT	3	2	0	5	0	10
MET	4	5	0	11	0	20
PPG	4	4	0	6	0	14

	Reasons for removal of cows from trial[3]						
Treatment	Mastitis	Retained Fetal Membrane	Displaced abomasum	Calved early	Died	Other	Total
CON	2	1	3	2	1	1	10
NUT	2	0	2	2	0	0	6
MET	4	1	0	0	2	0	7
PPG	3	0	1	1	0	0	5

[1] Treatments were: CON= no supplement; NUT= 113 g/d of NutroCal; MET= 454 g/d of Metaxerol; PPG = 300 mL/d of propylene glycol.
[2] Numbers represent number of incidences of health problems from 124 cows used in data analyses.
[3] Numbers represent cows (n=28) originally assigned to experiment but removed without providing sufficient data to be included in data analyses.

CHAPTER 4
SUMMARY AND CONCLUSIONS

Growth of heifers is a pliable function and can be modified to improve productivity. In spite of this, modifying growth rates has not yet led to substantial advances in replacement heifer management beyond reducing age at first calving (AFC). Therefore, the decision to reduce AFC should be a management decision which is herd specific.

Although feeding higher energy and/or protein diets increased growth rates through 341 kg of BW, no effects of diet were found on age, BW or BCS at first calving, number of breedings, or birth weight of calves. Increasing CP from 14% to 18% resulted in greater prepubertal ADG but had no effect on 150 d MY. Independent of diet, SEA, or bST injections, greater ADG were negatively correlated to 150 d MY.

Prepubertal injections of bST accelerated growth rates by only 3.96%. Injections had no effect on AFC, number of breedings, calving or mean postpartum BW and BCS, and importantly no effect on 150 d MY. Prepubertal injections of bST at the current dose seem not to be economically advantageous for improving either AFC or milk production. It is possible that the lack of growth response to bST injections could be diet-related, response of bST may differ as diets fed are changed.

In summary, there is potential in the use of bST and higher CP:ME ratio in the diet to improve heifer performance, but additional research is needed to determine the best feeding practices and bST treatments to potentially affect growth, AFC MY. Additionally, the extra cost of bST injections and/or improved diets should be considered when evaluating the economic feasibility of its use.

Supplementation of glucogenic precursors, at recommended rates, during the transition period of multiparous Holstein cows provoked no significant changes in their overall performance. It is important to indicate that the glucogenic supplements NUT and MET were fed as recommended by the respective manufacturer. The amount (300 mL/d) of PPG supplemented was chosen because literature showed this was an effective dose. The method of delivery of the glucogenic supplements by mixing them in the TMRs fed was chosen to better simulate practice likely to be used on commercial dairy farms. Current recommendations are to top-dress the supplement to daily feed offered. The absolute glucogenic potential could not be calculated for the three supplements and therefore direct comparisons could not be made. Nonetheless, specific responses were measured for supplements as fed to evaluate their potential benefits.

Dry matter intake was depressed similarly around calving in all treatment groups. Overall 150 d Milk yield of cows fed the supplements did not differ and only small differences were found in relative feed efficiencies (MY/DMI) during the first 4 wk postpartum.

The MY was greater for cows that calved during the cooler season (SEA 1) and percentage decreases during SEA 2 were 0.0%, 6.47%, 16.36%, and 18.64% less for cows in CON, NUT, MET, and PPG, respectively. Overall, hormonal trends did not differ among diet treatment groups, although some differences were found between treatments. Liver samples collected by biopsy showed no differences in percentage of fat on a wet wt basis due to treatments. Feeding the glucogenic supplements evaluated in this experiment showed no negative or positive effects when used at the recommended feeding rates. Although no economic or physiological advantages were found, additional research should be conducted on this topic with a greater number of cows to evaluate health benefits because this could be a major economic consideration even in the absence of significant effects of milk production.

APPENDIX

Table A. Treatment orthogonal contrasts of dry matter intake, body weight, body condition score and blood hormone, and growth factor and metabolites for Holstein cows fed glucogenic supplements during the transition period (-3 wk to +4 wk).[1]

	Treatment Contrasts					
	CON vs. NUT, MET, PPG		NUT, MET vs. PPG		NUT vs. MET	
Variable	Prepartum	Postpartum	Prepartum	Postpartum	Prepartum	Postpartum
Dry Matter Intake	P= 0.474	P= 0.219	P= 0.243	P= 0.929	P= 0.539	P= 0.160
Body Weight	P= 0.412	P= 0.053	P= 0.050	P= 0.764	P= 0.980	P= 0.230
Body Condition Score	P= 0.156	P= 0.516	P= 0.745	P= 0.214	P= 0.185	P= 0.020
Glucose	P= 0.563	P= 0.481	P= 0.938	P= 0.225	P= 0.756	P= 0.243
NEFA	P= 0.243	P= 0.256	P= 0.408	P= 0.027	P= 0.028	P= 0.166
β-HBA	P= 0.567	P= 0.275	P= 0.865	P= 0.054	P= 0.884	P= 0.117
Total Liver Fat	P= 0.696	P= 0.849	P= 0.998	P= 0.832	P= 0.749	P= 0.123
Insulin	P= 0.796	P= 0.529	P= 0.475	P= 0.095	P= 0.077	P= 0.842
IGF-I	P= 0.243	P= 0.189	P= 0.232	P= 0.013	P= 0.025	P= 0.846
Calcium	P= 0.144	P= 0.624	P= 0.115	P= 0.275	P= 0.738	P= 0.919

[1] Treatments were: CON= no supplement; NUT= 113 g/d of NutroCal; MET= 454 g/d of Metaxerol; PPG = 300 mL/d of propylene glycol.

Table B. Treatment orthogonal contrasts for milk yield and composition for Holstein cows fed glucogenic supplements during the transition period (-3 wk to +4 wk).[1]

	Contrasts (treatments)		
Variable	CON vs. NUT, MET, PPG	NUT, MET vs. PPG	NUT vs. MET
Milk Yield			
wk 1 to wk 4	P= 0.114	P= 0.028	P= 0.754
wk 5 to wk 10	P= 0.111	P= 0.157	P= 0.777
wk 11 to wk 15	P= 0.839	P= 0.742	P= 0.133
Milk Composition			
Somatic Cell Count	P= 0.202	P= 0.419	P= 0.671
Milk Fat	P= 0.337	P= 0.427	P= 0.831
Milk Protein	P= 0.556	P= 0.001	P= 0.084

[1] Treatments were: CON= no supplement; NUT= 113 g/d of NutroCal; MET= 454 g/d of Metaxerol; PPG = 300 mL/d of propylene glycol.

REFERENCES

Abeni, F., L. Calamari, L. Stefanini, and G. Pirlo. 2000. Effects of daily gain in pre- and postpubertal replacement dairy heifers on body condition score, body size, metabolic profile, and future milk production. J. Dairy Sci. 83:1469-1478.

Abribat, T.H., H. Lapierre, P. Dubreuil, G. Pelletier, P. Gaudreau, P. Brazeau, and D. Petitclerc. 1990. Insulin-like growth factor-1 concentration in Holstein female cattle: variations with age, stage of lactation, and growth hormone-releasing factor administration. Domest. Anim. Endocrinol. 7:93-102.

Akers, R.M., T.B. McFadden, S. Purup, M. Vestergaard, K. Sejrsen, and A.V. Capuco. 2000. Local IGF-1 in peripubertal ruminant mammary development. J. Mammary Gland Bio. Neoplasia 5:43-51.

Akinyode, A., M.B. Hall, J.P. Jennings, C.R. Staples, and C.J. Wilcox. 2001. Effects of feeding different sources of neutral detergent-soluble carbohydrates supplemented with fat and propionate to heat stressed dairy cows. J. Dairy Sci. 84 (Suppl. 1):121 (Abstr.).

Allen, M.S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598-1624.

Annesxtad, J. 1986. Raise replacements economically. Dairy Herd Magazine. 23:20-22.

Annison, E.F. and D.G. Armstrong. 1970. Volatile fatty acid metabolism and energy supply. In: Physiology of Digestion and Metabolism in the Ruminant. Phillipson, A. T., Ed. p. 422. Oriel Press. Newcastle upon Tyne, England.

Anonymous. 2003. β-hydroxybutyrate reagent kit flyer. Pointe Scientific, Inc. Detroit, MI.

Arieli, A., J.E. Vallimont, Y. Aharoni, and G.A. Varga. 2001. Monensin and growth hormone effects on glucose metabolism in the prepartum cow. J. Dairy Sci. 84:2770-2776.

Armentano, L.E., R.R. Grummer, S.J. Bertics, T.C. Skaar, and S.S. Donkin. 1991. Effect of energy balance on hepatic capacity for oleate and propionate metabolism and triglyceride secretion. J. Dairy Sci. 74:132-139.

Bagg, J.G., D.G. Grieve, J.H. Burton, and J.B. Stone. 1985. Effect of protein on growth of Holstein heifer calves from 2 to 10 months. J. Dairy Sci. 68:2929-2939.

Baird, G.D., A. Lomax, H.W. Symonds, and S.R. Shaw. 1980. Net hepatic and splanchnic metabolism of lactate, pyruvate and propionate in dairy cows in vivo in relation to lactation and nutrient supply. Biochem. J. 186:47-57.

Bauman, D.E. 1992. Bovine somatotropin: review of an emerging animal technology. J. Dairy Sci. 75:3432-3452.

Bauman, D.E., and R.G. Vernon. 1993. Effects of exogenous bovine somatotropin on lactation. Ann. Rev. Nutr. 13:437-461.

Beem, A.E., H.G. Bateman, C.C. Williams, C.C. Stanley, D.T. Gantt, Y.H. Chung, and F.R. Valdez. 2003. Effects of prepartum dietary energy concentration and Ca-propionate on transition performance. J. Dairy Sci. 86 (Suppl.1):105 (Abstr.).

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804-2819.

Bergman, E.N. 1990. Energy contribution of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70(2):567-590.

Bethard, G.L., R.E. James, and M.L. Mcgilliard. 1997. Effect of rumen-undegradable protein and energy on growth and feed efficiency of growing Holstein heifers. J. Dairy Sci. 80:2149-2155.

Bertics, S.J., R.R. Grummer, C. Cadorniga-Valino, and E.E. Stoddard. 1992. Effect of prepartum dry matter intake on liver triglyceride concentration and early lactation. J. Dairy Sci. 75:1914-1922.

Bragg, R. 1997. Mode of action of ionophores in lactating dairy cattle. Pp. 13–21 in Usefulness of Ionophores in Lactating Dairy Cattle. Symp. Ontario Vet. College, Geulph, Canada.

Breier, B.H., J.J. Bass, J.H. Butler, and P.D. Gluckman. 1986. The somatotrophic axis in young steers: influence of nutritional status on pulsatile release of growth hormone and circulating concentrations of insulin-like growth factor-1. J. Endocrinol. 111:209-216.

Bremmer, D.R., S.L. Trower, S.J. Bertics, S.A. Besong, U. Bernabucci, and R.R. Grummer. 2000. Etiology of fatty liver in dairy cattle: effects of nutritional and hormonal status on hepatic microsomal triglyceride transfer protein. J. Dairy Sci. 83: 2239-2251.

Broster, W.H., and V.J. Broster. 1998. Body score of dairy cows. J. Dairy Res. 155-161.

Bruss, M.L. 1993. Metabolic fatty liver of ruminants. Adv. Vet. Sci. Comp. Med. 37:417–449.

Bucklin, R.A., G.L. Hahn, D.K. Beede, and D.R. Bray. 1992. Physical facilities for warm climates. In: Large dairy herd management. Van Horn, H.H., and C.J. Wilcox. (Ed.) pp. 609-618.

Burhans, W.S., and A. W. Bell. 1998. Feeding the transition cow. Proceedings 1998 Cornell Nutrition Conference, Ithaca, NY, p. 247-257.

Burhans, W.S., E.A. Briggs, J.A. Rathmacher, and A.W. Bell. 1997. Glucogenic supplementation does not reduce body tissue protein degradation in prepubertal dairy cows. J. Dairy Sci. 80(Suppl. 1):166 (Abstr.).

Capuco, A.V., J.J. Smith, D.R. Waldo, and T.H. Elsasser. 1988. Effect of diet and prepubertal growth rate of Holstein heifers on mammary gland growth and milk production. J. Dairy Sci. 71(Suppl. 1):229 (Abstr.).

Capuco, A. V., J.J. Smith, D.R. Waldo, and C.E. Rexroad, Jr. 1995. Influence of prepubertal dietary regimen on mammary growth of Holstein heifers. J. Dairy Sci. 78:2709-2725.

Capuco, A. V., D.L. Wood, R. Baldwin, K. McLeod, and M.J. Paape. 2001. Mammary cell number, proliferation, and apoptosis during a bovine lactation: relation to milk production and effect of bST. J. Dairy Sci. 84:2177-2187.

Christensen, J.O., R.R. Grummer, F.E. Rasmussen, and S.J. Bertics. 1997. Effect of method of delivery of propylene glycol on plasma metabolites of feed-restricted cattle. J. Dairy Sci. 80:563-568.

Correa, M.T., C.R. Curtis, H. N. Erb, J. M. Scarlett, and R. D. Smith. 1990. An ecological analysis of risk factors for postpartum disorders of Holstein-Friesian cows from thirty-two New York farms. J. Dairy Sci. 73:1515-1524.

Curtis, C.R., H.N. Erb, C.J. Sniffen, R.D. Smith, and D.S. Kronfeld. 1985. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. J. Dairy Sci. 68:2347-2360.

Daccarett, M.G., E.J. Bortone, D.E. Isbell, J.L. Morrill, and A.M. Feyerherm. 1993. Performance of Holstein heifers fed 100% or more of National Research Council requirements. J. Dairy Sci. 76:606–614.

Dahl, G.E., L.T. Chapin, M. S. Allen, W. M. Moseley, and H. A. Tucker. 1991. Comparison of somatotropin and growth hormone-releasing factor on milk yield, serum hormones, and energy status. J. Dairy Sci. 74: 3421-3428.

Daniel, L.R., B.P. Chew, T.S. Tanaka, and L.W. Tjoelker. 1991. Beta-carotene and vitamin A effects on bovine phagocyte function in vitro during the peripartum period. J. Dairy Sci. 74: 124-131.

Daughaday, E., and P. Rotwein. 1989. Insulin like growth factor I and II. Peptide, messenger ribonucleic acid and gene structure, serum and tissue concentrations. Endocrin. Rev. 10: 68-91.

Dobos, R.C., K.S. Nandra, K. Riley, W.J. Fulkerson, I.J. Lean, and R.C. Kellaway. 2000. The effect of dietary protein level during the pre-pubertal period of growth on mammary gland development and subsequent milk production in Friesian heifers. Livestock Prod. Sci. 63:235-243.

Drackley, J.K. 1992. Niacin and carnitine in the nutrition of dairy cows. Pp. 8–21 in Proc. Pacific Northwest Nutr. Conf. Tech. Symp., Spokane, WA. Lonza Inc., Fair Lawn, NJ.

Drackley, J.K. 2003. Nutritional management of the transition cow. http://www.txanc.org/proceedings/2001/NutritionalManagementTransitionCows.pdf.

Drackley, J.K., D.W. LaCount, J.P. Elliott, T.H. Klusmeyer, T.R. Overton, J.H. Clark, and S.A. Blum. 1998. Supplemental fat and nicotinic acid for Holstein cows during an entire lactation. J. Dairy Sci. 81:201-214.

Drackley, J.K., M.J. Richard, D.C. Beitz, and J.W. Young. 1992. Metabolic changes in dairy cows with ketonemia in response to feed restriction and dietary 1,3-butanediol. J. Dairy Sci. 75:1622-1634.

Drackley, J.K., and D.J. Schingoethe. 1986. Extruded blend of soybean meal and flower seeds for dairy cattle in early lactation. J. Dairy Sci. 69:371.

Duffield, T.F., D.F. Kelton, K.E. Leslie, K.D. Lissemore, and J.H. Lumsden. 1997. Use of test day milk fat and milk protein to detect subclinical ketosis in dairy cattle in Ontario. Can. Vet. J. 38:713–718.

Duffield, T.F., S. LeBlanc, R. Bagg, K. Leslie, J. Ten Hag, and P. Dick. 2003. Effect of a monensin controlled release capsule on metabolic parameters in transition dairy cows. J. Dairy Sci. 86: 1171-1176.

Duffield, T. F., D. Sandals, K. L. Leslie, K. Lissemore, B. W. McBride, J. H. Lumsden, P. Dick, and R. Bagg. 1998a. Effect of prepartum administration of monensin in a controlled-release capsule on postpartum energy indicators in lactating dairy cows. J. Dairy Sci. 81:2354–2361.

Duffield, T. F., D. Sandals, K. L. Leslie, K. Lissemore, B. W. McBride, J. H. Lumsden, P. Dick, and R. Bagg. 1998b. Efficacy of monensin for the prevention of subclinical ketosis in lactating dairy cows. J. Dairy Sci. 81:2866–2873.

Elliott, J. P., J. K. Drackley, D. J. Schauff, and E. H. Jaster. 1993. Diets containing high corn oil and tallow for dairy cows during early lactation. J. Dairy Sci. 76:775–789.

Emery, R.S. 1993. Energy needs of dry cows. Page 35. In: Proc. Tri-State Dairy Nutr. Conf. Ohio State Univ., Michigan State Univ., and Purdue Univ., Ft. Wayne, IN.

Emery, R.S., R.E. Brown, and A.L. Black. 1967. Metabolism of DL-1,2-propanediol-2-^{14}C in a lactating cow. J. Nutr. 92:348-356.

Emery, R.S., J.S. Liesman, and T.H. Hardt. 1992. Metabolism of long chain fatty acids by ruminant liver. J. Nutr. 122(Suppl. 3):832-837.

Endecott, R.L., D.L. Dunlap, R.C. Waterman, A.C. Fitzgerald, V A. Munn, C.A. Loest, D.E. Hawkins, K.K. Kane, F. Valdez, and M.K. Petersen. 2003. Increasing glucogenic precursors in range supplements fed to young postpartum beef cows. J. Dairy Sci. 86 (Suppl. 1): 212 (Abstr.).

Enjalbert, F., M.C. Nicot, C. Bayourthe, and R. Moncoulon. 2000. Effects of duodenal infusions of palmitic, stearic, or oleic acids on milk composition and physical properties of butter. J. Dairy Sci. 83: 1428-1433.

Enright, W.J. 1989. Effects of administration of somatotropin on growth, feed efficiency and carcass composition of ruminants: a review. In: Use of somatotropin in livestock production. K. Sejrsen, M. Vestergaard, and A. Neimann-Sorensen (Ed.) Elsevier Appl. Sci., London, England, pp. 132-156.

Eppard, P. J., D. E. Bauman, C. R. Curtis, H. N. Erb, G. M. Lanza, and M. J. DeGeeter. 1987. Effect of 188-day treatment with somatotropin on health and reproductive performance of lactating dairy cows. J. Dairy Sci. 70:582-591.

Eppard, P.J., S. Hudson, W.J. Cole, R.L. Hintz, G.F. Hartnell, T.W. Hunter, L.E. Metzger, A.R. Torkelson, B.G. Hammond, R.J. Collier, and O.M. Lanza. 1991. Response of dairy cows to high doses of a sustained-release bovine somatotropin administered during two lactations. 1. Production response. J. Dairy Sci. 74: 3807-3821.

Erb, H.N., and Y.T. Grohn. 1 988. Epidemiology of metabolism disorders in the periparturient dairy cow. J. Dairy Sci. 71:2557–2571.

Ferguson, J.D., D.T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci. 77:2695-2703.

Finn, A.M., A.K. Clark, J.K. Drackley, D.J. Schingoelhe. and T. Sahlu. 1985. Whole rolled sunflower seeds with or without additional limestone in lactating dairy cattle rations. J. Dairy Sci. 68:903-913.

Fisher, L.J., J.D. Erfel, and F.D. Sauer. 1971. Inducement of ketotic symptoms in lactating cows by reducing their plane of nutrition. Can J. Anim. Sci. 51:153-160.

Foldager, J., and K. Sejrsen. 1982. Nutrition of replacement heifers affects mammary development and their ability to produce milk. Pp. 451–457 in Proc. XIIth World Congr. Diseases Cattle. Vol. 1. World Assoc. Buiatrics, Utrecht, The Netherlands.

Foldager, J., and K. Sejrsen. 1991. Rearing intensity in dairy heifers and the effect on subsequent milk production. Rep. Natl. Inst. Anim. Sci. No. 693. Natl. Inst. Anim. Sci., Tejle, Denmark.

Forbes, M.J. 1995. Voluntary dry matter intake: a limiting factor to production in high yielding dairy cows? Br. Soc. Anim. Sci. Occ. Publ. 19:13-19.

Forbes, M.J. 1996. Integration of regulatory signals controlling forage intake in ruminants. J. Anim. Sci. 74:3029-3035.

Franklin, S.T., J.W. Young, and B.J. Nonnecke. 1991. Effects of ketones, acetate, butyrate, and glucose on bovine lymphocyte proliferation. J. Dairy Sci. 74:2507-2514.

Fuentes-Pila, J., M.A. DeLorenzo, D.K. Beede, C.R. Staples, and J.B. Holter. 1996. Evaluation of equations based on animal factors to predict intake of lactating Holstein cows. J. Dairy Sci. 79:1562-1571.

Fuentes-Pila, J., M. Ibanez, J.M. De Miguel, and D.K. Beede. 2003. Predicting average feed intake of lactating Holstein cows fed totally mixed rations. J. Dairy Sci. 86:309-323.

Gabler, M.T., and A.J. Heinrichs. 2003. Dietary protein to metabolizable energy ratios on feed efficiency and structural growth of prepubertal Holstein heifers. J. Dairy Sci. 86:268-274.

Gabler, M.T., P.R. Tozer, and A.J. Heinrichs. 2000. Development of a cost analysis spreadsheet for calculating the costs to raise a replacement dairy heifer
J. Dairy Sci. 83:1104-1109.

Garcia-Gavidia, A.N. 1998. Use of bovine somatotropin (bST) in management of growing heifers and transition cows to improve growth rates and milk production. PhD. Dissertation. University of Florida.

Gardner, R.W., J.D. Schuh, and L.G. Vargus. 1977. Accelerated growth and early breeding of Holstein heifers. J. Dairy Sci. 60:1941-1946.

Gardner, R. W., L. W. Smith, and R. L. Park. 1988. Feeding and management of dairy heifers for optimal lifetime productivity. J. Dairy Sci. 71:996-999.

Geishauser, T.K. Leslie, D. Kelto, and T. Duffield. 1998. Evaluation of five cowside tests for use with milk to detect subclinical ketosis in dairy cows. J. Dairy Sci. 81:438–443.

Gerloff, B.J., T.H. Herdt, and R.S. Emery. 1986. Relationship of hepatic lipidosis to health and performance in dairy cattle. JAVMA 188:845–850.

Goff, J.P., T.F. Brown, S.R. Stokes, C.L. Brawley, and F.R. Valdez. 2002. Titration of the proper dose of calcium propionate (NutroCAL) to be included in an oral drench for fresh cows. J. Dairy Sci. 85(Suppl. 1):189. (Abstr.).

Goff, J. P., and R. L. Horst. 1997. Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci. 80:1260–1268.

Goff, J.P., and R.L. Horst. 2001. Oral glycerol as an aid in the treatment of ketosis/fatty liver complex. J. Dairy Sci. 84(Suppl. 1):153. (Abstr.).

Goff, J. P., R.L. Horst, P.W. Jardon, C. Borelli, and J. Wedam. 1996. Field trials of an oral calcium propionate paste as an aid to prevent milk fever in periparturient dairy cows. J. Dairy Sci. 79:378-383.

Goff J.P., and J. R. Stabel. 1990. Decreased plasma retinol, alpha-tocopherol, and zinc concentration during the periparturient period: effect of milk fever. J. Dairy Sci. 73:3195-3199.

Goodger, W.J., J. Fetrow, G.M. Ferguson, H.F. Troutt, and R. McCabe. 1989. A computer spreadsheet program to estimate the cost of raising dairy replacments. Prev. Vet. Med. 7:239-246.

Grant, R.J., and J.L. Albright. 1995. Feeding behavior and management factors during the transition period in dairy cattle. J. Anim Sci. 73:2791-2803.

Green, B.L., B.W. McBride, D. Sandals, K.E. Leslie, R. Bagg, and P. Dick. 1999. The impact of a monensin controlled-release capsule on subclinical ketosis in the transition dairy cow. J. Dairy Sci. 82:333-342.

Grings, E.E., D.M. de Avila, R.G. Eggert, and J.J. Reeves. 1990. Conception rate, growth, and lactation of dairy heifers treated with recombinant somatotropin. J. Dairy Sci. 73:73-80.

Grum, D.E., J.K. Drackley, L.R. Hansen, and J.D. Cremin, Jr. 1996. Production, digestion and hepatic lipid metabolism of dairy cows fed increased energy from fat or concentrate. J. Dairy Sci. 79:1836-1849.

Grummer, R.R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76:3882–3896.

Grummer, R.R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J. Anim. Sci. 73:2820-2833.

Grummer, R.R., and D.J. Carroll. 1991. Effects of dietary fat on metabolic disorders and reproductive performance of dairy cattle. J. Anim. Sci. 69:3838–3852.

Grummer, R.R., J. C. Winkler, S.J. Bertics, and V.A. Studer. 1994. Effect of propylene glycol dosage during feed restriction on metabolites in blood of prepartum Holstein heifers. J. Dairy Sci. 77:3618-3623.

Havel, R.J. 1987. Lipid transport function of lipoproteins in blood plasma. Am. J. Physiol. 253(Endocrinol. Metab. 16):E1-E5.

Hayirli, A., R.R. Grummer, E.V. Nordheim, and P.M. Crump. 2002. Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. J. Dairy Sci. 85:3430-3443.

Head, H.H., K.C. Bachman, M. Campos, and C. J. Wilcox. 1991. Effects of plane of nutrition and somatotropin on growth rates and first lactation milk yields of Holstein heifers. J. Dairy Sci. 74(Suppl. 1):189 (Abstr.).

Head, H.H., Liboni, M., and T.I. Belloso. 2002. The association of accelerated growth rates of heifers and milk yields: problems and opportunities. Pp.31-41 In:
Proceedings of the Southeast Replacement Dairy Heifer Workshop and Field Day. Gainesville, FL.

Heinrichs, A. J. 1993. Raising dairy replacements to meet the needs of the 21st century. J. Dairy Sci. 76:3179-3184.

Heinrichs, A. J., and L.A. Swartz. 1990. Management of dairy heifers. Pennsylvania State Univ. Ext Circ. 385, University Park.

Herdt, T.H. 1988. Fatty liver in dairy cows. Vet. Clin. North Am. Food Anim. Pract. 4:269–287.

Heuer, C. 2000. Negative energy balance in dairy cows- prediction, consequence, prevention. Ph.D. thesis, Utrecht University.

Hoffman, P.C. 1997. Optimum body size of Holstein replacement heifers. J. Anim. Sci. 75:836-845.

Hoffman, P.C. 2002. Contemporary issues in applied dairy replacement heifer research. J. Dairy Sci. 85(Suppl 1):79.

Hoffman, P.C., N.M. Brehm, S.G. Price, and A. Prill-Adams. 1996. Effect of accelerated postpubertal growth and early calving on lactation performance of primiparous Holstein heifers. J. Dairy Sci. 79:2024-2031.

Hoffman, P.C., and D.A. Funk. 1992. Applied dynamics of dairy replacement growth and management. J. Dairy Sci. 75:2504-2511.

Hogan, J.S., W. P. Weiss, and K. L. Smith. 1993. Role of vitamin E and selenium in host defense against mastitis. J. Dairy Sci. 76:2795-2803.

Holter, J.B., M.J. Slotnick, H.H. Hayes, C.K. Bozak, W.E. Urban, Jr, and M.L. McGilliard. 1990. Effect of prepartum dietary energy on condition score, postpartum energy, nitrogen partitions, and lactation production responses. J. Dairy Sci. 73:3502-3511.

Hoover, W.H., and S.R. Stokes. 1991. Balancing carbohydrate and protein for optimum rumen microbial yield. J. Dairy Sci. 74:3630–3644.

Huff, E. 1961. Metabolism of 1,2-propanediol. Bioch. Biophy. Acta. 48: 506-509.

Ingvartsen, K.L. 1994. Models of voluntary food intake in cattle. Livest. Prod. Sci. 39:19-38.

Ingvartsen, K.L., and J. B. Andersen. 2000. Integration of metabolism and intake regulation: a review focusing on periparturient animals. J. Dairy Sci. 83: 1573-1597.

Ingvartsen, K.L., J. B. Andersen, and J. Foldager. 1992. Effect of sex and pregnancy on feed intake capacity of growing cattle. Acta Agric. Scand. A, Anim. Sci. 42:40-46.

Jerred, M.J., D.J. Carroll, D.K. Combs, and R.R. Grummer. 1990. Effect of fat supplementation and immature alfalfa to concentrate ratio on lactation performance of dairy cows. J. Dairy Sci. 73:2842-2854.

Johnson, M.M, and J.P. Peters. 1993. Technical note: an improved method to quantify nonesterified fatty acids in bovine plasma. J. Anim. Sci. 71:753-756.

Johnson, N.N., R.C.W. Daniel, D. Mayer, and R. Verral. 1998. Effects of oral dosing with calcium propionate on total calcium and glucose concentrations in the plasma of the cow. J. Vet. Med. Series A- Phys. Path. Clin. Med. 45:127-136.

Kertz, A.F., L.R. Prewitt, and J.M. Ballam. 1987. Increased weight gain and effects on growth parameters of Holstein heifer calves from 3 to 12 months of age. J. Dairy Sci. 70:1612-1622.

Kertz, A.F., L.F. Reutzel, and G. M. Thomson. 1991. Dry matter intake from parturition to midlactation. J. Dairy Sci. 74:2290-2295.

Keown, J.F., and R.W. Everett. 1986. Effect of days carried calf, days dry, and weight of first calf heifers on yield. J. Dairy Sci. 69:1891–1896.

Khorasani, G.R., G. de Boer, P.H. Robinson, and J.J. Kennelly. 1992. Effect of canola fat on ruminal and total tract digestion, plasma hormones, and metabolites in lactating dairy cows. J. Dairy Sci. 75:492-501.

Kronfeld, D. S. 1982. Major metabolic determinants of milk volume, mammary efficiency, and spontaneous ketosis in dairy cows. J. Dairy Sci. 65:2204-2212.

Lammers, B.P., and A.J. Heinrichs. 2000. The response of altering the ratio of dietary protein to energy on growth, feed efficiency, and mammary development in rapidly growing prepubertal heifers. J. Dairy Sci. 83:977-983.

Lammers, B.P., A.J. Heinrichs, and R.S. Kensinger. 1999. The effects of accelerated growth rates and estrogen implants in prepubertal Holstein heifers on growth, efficiency and blood parameters. J. Dairy Sci. 82:1746-1752.

Littell, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 2000. SAS systems for mixed models. SAS institute Inc., Cary, NC.

Little, W., and R.M. Kay. 1979. The effects of rapid rearing and early calving on the subsequent performance of dairy heifers. Anim. Prod. 29:131-142.

Malven, P.V., H.H. Head, R.J. Collier, and F.C. Buonomo. 1987. Periparturient changes in the secretion and mammary uptake of insulin and in concentrations of insulin-like growth factors in milk of dairy cows. J. Dairy Sci. 70:2254-2265.

Mantysaari, P.E., C.J. Sniffen, T.V. Muscato, and M.L. Thonney. 1989. Performance of growing dairy heifers fed diets containing soybean meal or animal by-product meals. J. Dairy Sci. 72:2107-2114.

McGarry, J.D., and Brown, N.F. 1997. The mitochondrial carnitine palmitoyl-transferase system. From concept to molecular analysis. Eur. J. Biochem. 244: 1-14.

McGuire, M.A., J.L. Vicini, D.E. Bauman, and J.J. Veenhuizen. 1992. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. J. Anim. Sci. 70:2901-2910.

McShane, T. M., K.K. Schillo, J.A. Boling, N.W. Bradley, and J.B. Hall. 1989. Effects of recombinant DNA-derived somatotropin and dietary energy intake in development of beef heifers: I. Growth and puberty. J. Anim. Sci. 67:2230-2236.

Melendez, P., A. Donovan, C.A. Risco, M.B. Hall, R. Littell, and J. Goff. 2002. Metabolic responses of transition Holstein cows fed anionic salts and supplemented at calving with calcium and energy. J. Dairy Sci. 85:1085-1092.

Miles, P.H., N.S. Wilkinson, and L.R. McDowell. 2001. Analysis of minerals for animal nutrition research. 3rd Ed. Department of Animal Sciences, University of Florida, Gainesville, FL.

Miller, O.N., and G. Bazzano. 1965. Propanediol metabolism and its relation to lactic acid metabolism. Ann. New York Acad. Sci. 119:957–973.

Morbeck, D.E., J.H. Britt, and B.T. McDaniel. 1991. Relationships among milk yield, metabolism, and reproductive performance of primiparous Holstein cows treated with somatotropin. J. Dairy Sci. 74: 2153-2164.

Moseley. W.M., J.B. Paulissen, M.C. Goodwin, G.R. Alaniz, and W.H. Claflin. 1992. Recombinant bovine somatotropin improves growth performance in finishing beef steers. J. Anim. Sci. 70:412-425.

Murphy, K.D., D.G. Johnson, R.D. Appleman, and D.E. Otterby. 1991. Effects of rearing diet, age at freshening, and lactation feeding system on performance.
J. Dairy Sci. 74:2708-2717.

Muylle, E., C. Van den Hende, B. Sustronck, and P. Deprez. 1990. Biochemical profiles in cows with abomasal displacement estimated by blood and liver parameters. J. Vet. Med. Ser. A. 37:259–263.

National Research Council. 1989. Nutrient requirements of dairy cattle. 6th Rev. Ed. Natl. Acad. Sci., Washington, DC.

National Research Council. 2001. Nutrient requirements of dairy cattle. 7th Rev. Ed. Natl. Acad. Sci., Washington, DC.

Newsholme, E.A., B.F. Crabtree, and M.S.M. Ardawi. 1985. Glutamine metabolism in lymphocytes: its biochemical, physiological and clinical importance. Quarterly J. of Exp. Phy. 70:473-489.

Nocek, J.E. 1995. Nutritional aspects of the transition cow. Pp. 121–137 in Proc. Cornell Nutr. Conf. Feed Manuf. Ithaca, NY.

Nolan, J.P. 1989. Intestinal endotoxins as mediators of hepatic injury-an idea whose time has come again. Hepatology 10: 887-891.

Oldham, J.D. 1984. Protein-energy interrelationships in dairy cows. J. Dairy Sci. 67:1090-1114.

Overton, T.R. 2000. NEFA and BHBA testing for transition cows. http://www.ansci.cornell.edu/dm/nutrition/tdn00/tdnsep00.htm Accessed Dec. 2003.

Overton, T.R. 2003. Transition cows program – Management and metabolism. http://animal.cals.arizona.edu/swnmc/pdf/2002/Overton2002.pdf Accessed Dec. 2003

Overton, T.R., J.K. Drackley, G.N. Douglas, L.S. Emmert, and J.H. Clark. 1998.
Hepatic gluconeogenesis and whole-body protein metabolism of periparturient dairy cows as affected by source of energy and intake of the prepartum diet. J. Dairy Sci. 81(Suppl. 1):295 (Abstr.).

Overton, T.R., J.K. Drackley, C.J. Ottemann-Abbamonte, A.D. Beaulieu, L.S. Emmert, and J. H. Clark. 1999. Substrate utilization for hepatic gluconeogenesis is altered by increased glucose demand in ruminants. J. Anim. Sci. 77:1940– 1951.

Overton, T.R., L.S. Emmert, and J.H. Clark. 1998. Effects of source of carbohydrate and protein and rumen-protected methionine on performance of cows. J. Dairy Sci. 81:221-228.

Park, C. S., G. M. Erickson, Y. J. Choi, and G. D. Marx. 1987. Effect of compensatory growth on regulation of growth and lactation: response of dairy heifers to a stair-step growth pattern. J. Anim. Sci. 64:1751-1758.

Pickett, M.M., M.S. Piepenbrink, and T.R. Overton. 2003. Effects of propylene glycol or fat drench on plasma metabolites, liver composition, and production of dairy cows during the periparturient period. J. Dairy Sci. 86:2113-2121.

Pirlo, G., M. Capelleti, and G. Marchetto. 1997. Effect of energy and protein allowances in the diets of prepubertal heifers on growth and milk production. J. Dairy Sci. 80:730-739.

Pirlo, G., F. Miglior, and M. Speroni. 2000. Effect of age at first calving on production traits and on difference between milk yield returns and rearing costs in Italian Holsteins. J. Dairy Sci. 83:603-608.

Preston, R.L. 1966. Protein requirements of growing-finishing cattle and lambs. J. Nutr. 90:157-160.

Pullen, D.L., D.L. Palmquist, and R.S. Emery. 1989. Effect on days of lactation and methionine hydroxy analog on incorporation of plasma fatty acids into plasma triglycerides. J. Dairy Sci. 72:49–58.

Purup, S., K. Sejrsen, and R.M. Akers. 1995. Effect of bovine GH and ovariectomy on mammary tissue sensitivity to IGF-I in prepubertal heifers. J. Endocrinol. 144:153–158.

Purup, S., K. Sejrsen, J. Foldager, and R.M. Akers. 1993. Effect of exogenous bovine growth hormone and ovariectomy on prepubertal mammary growth, serum hormones and acute in vitro proliferative response of mammary explants from Holstein heifers. J. Endocrinol. 139:19-26.

Quigley, J.D., and J.J. Drewry. 1998. Nutrient and immunity transfer from cow to calf pre-and post-calving. J. Dairy Sci. 81:2779-2790.

Raabo, E., and T.C. Terkildsen. 1960. The enzymatic determination of blood glucose. Scand. J. Clin. Lab. Invest. 12:402-408.

Radcliff, R.P., M.J. VandeHaar, L.T. Chapin, T.E. Pilbeam, D.K. Beede, E.P. Stanisiewski, and H.A. Tucker. 2000. Effects of diet and injection of bovine somatotropin on prepubertal growth and first-lactation milk yields of Holstein cows. J. Dairy Sci. 83:23-29.

Radcliff, R.P., M.J. VandeHaar, A.L. Skidmore, L.T. Chapin, B.R. Radke, J.W. Lloyd, E.P. Stanisiewski, and H.A. Tucker. 1997. Effect of diet and bovine somatotropin on heifer growth and mammary development. J. Dairy Sci. 80:1996-2003.

Reid, I.M. 1982. Fatty liver in dairy cows- incidence, severity, pathology and functional consequences. Bov. Pract. 17:149-150.

Reid, I.M., A.M. Dew, and L.A. Williams. 1984. Haematology of subclinical fatty liver in dairy cows. Res. Vet. Sci. 37:63-66.

Reid, I.M., and C.J. Roberts. 1982. Fatty liver in dairy cows. Practice. 4:164–169.

Reid, I.M., C.J. Roberts, and R. Manston. 1979. Fatty liver and infertility in high
yielding dairy cows. Vet. Rec. 104:75-76.

Roseler, D.K., D.G. Fox, A.N. Pell, and L.E. Chase. 1997. Evaluation of alternative equation for prediction of intake for Holstein dairy cows. J. Dairy Sci. 80:864-877.

Rukkwamsuk, T., T. Wensing, and M.J. Geelen. 1999. Effect of fatty liver on hepatic gluconeogenesis in periparturient dairy cows. J. Dairy Sci. 82:500-505.

Russell, J.B., and R.B. Hespell. 1981. Microbial rumen fermentation. J. Dairy Sci. 64:1153–1171.

Sandles, L.D., and C.J. Peel. 1987. Mammogenesis and first lactation milk yields of identical-twin heifers following pre-pubertal administration of bovine growth hormone. Anim. Prod. 45:349-357.

Santos, F.A., J.E. Santos, C.B. Theurer, and J.T. Huber. 1998. Effects of rumen-undegradable protein on dairy cow performance: a 12-year literature review. J. Dairy Sci. 81:3182-3213.

SAS user's guide, Version 6, Release 6.07. 1993. SAS Inst., Inc., Cary, NC.

Schillo, K.K., J.B. Hall, and S.M. Hileman. 1992. Effect of nutrition and season on the onset of puberty in the beef heifer. J. Anim. Sci. 70:3994–4005.

Schultz, L.H. 1958. Use of sodium propionate in the prevention of ketosis in dairy cattle. J. Dairy Sci. 41:160-167.

Schurman, E.W., and E.M. Kesler. 1974. Protein-to-energy ratios in complete feeds for calves at ages 8 to 18 weeks. J. Dairy Sci. 57:1381–1384.

Sejrsen, K. 1978. Mammary development and milk yield in relation to growth rate in dairy and dual-purpose heifers. Acta Agric. Scand. 28:41-46.

Sejrsen, K. 1994. Relationships between nutrition, puberty and mammary development in cattle. Proc. Nut. Soc. 53:103-111.

Sejrsen, K., J. Foldager, M.T. Sorensen, R.M. Akers, and D.E. Bauman. 1986. Effect of exogenous bovine somatotropin on pubertal mammary development in heifers. J. Dairy Sci. 69:1528-1535.

Sejrsen, K., J.T. Huber, H.A. Tucker, and R.M. Akers. 1982. Influence of nutrition on mammary development in pre- and postpubertal heifers. J. Dairy Sci. 65:793-800.

Sejrsen, K., and S. Purup. 1997. Influence of prepubertal feeding level on milk yield potential of dairy heifers: a review J. Anim. Sci. 75:828-835.

Sejrsen, K., S. Purup, M. Vestergaard, and J. Foldager. 2000. High body weight gain and reduced bovine mammary growth: physiological basis and implications for milk yield potential. Domest. Anim. Endocrinol. 19:93–104.

Silva, L.F.P., M.J. VandeHaar, B.K. Whitlock, R.P. Radcliff, and H.A. Tucker. 2002. Short communication: relationship between body growth and mammary development in dairy heifers. J. Dairy Sci. 85:2600-2602.

Sinha, Y. N., and H. A. Tucker. 1969. Mammary development and pituitary prolactin levels of heifers from birth through puberty and during the estrous cycle. J. Dairy Sci. 52:507-512.

Smith, K.L., J.H. Harrison, D.D. Hancock, D.A. Todhunter, and H.R. Conrad. 1984. Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical symptoms. J. Dairy Sci. 67:1293-1300.

Soeldner, J.S., and D. Sloane. 1965. Critical variables in the radioimmunoassay of serum insulin using the double antibody technique. Diabetes 14:771-779.

Sorensen, C.E., A.R. Hippen, D.J. Schjingoethe, and R.S. Patton. 2001. Rumen inert lipids and glucose precursors lessen prepartum feed intake depression and improve carbohydrate status in periparturient dairy cows. J. Dairy Sci. 84 (Suppl 1):10(Abstr.).

Sorensen, C.E., R.S. Patton, and A.R. Hippen. Unpublished, personal communication with R.S. Patton. Precalving dry matter intake influenced in Holstein cows by dietary supplement of glucogenic precursors and fat but not fat alone.

Spears, J.W., V. Fellner, and F.R. Valdez. 2002. Comparative metabolism of calcium from calcium carbonate and calcium propionate in growing steers. J. Dairy Sci. 85 (Suppl. 1): 366 (Abstr.).

Spicer, L.J., W.B. Tucker, and G.D. Adams. 1990. Insulin-like growth factor-I in dairy cows: relationships among energy balance, body condition, ovarian activity, and estrous behavior. J. Dairy Sci. 73:929-937.

Stabel, J.R., T.A. Reinhardt, M.A. Stevens, M.E. Kehrli, Jr, and B.J. Nonnecke. 1992. Vitamin E effects on in vitro immunoglobulin M and interleukin-1 beta production and transcription in dairy cattle. J. Dairy Sci. 75:2190-2198.

Steinhour, W.D., and D.E. Bauman. 1986. Propionate metabolism: a new interpretation. Pp. 238 In: Aspects of digestive physiology in ruminants. Alan Dobson and Marjorie Dobson, eds. Cornell Univ. Press, Ithaca.

Stelwagen, K., and D. G. Grieve. 1990. Effect of plane of nutrition on growth and mammary development in Holstein heifers. J. Dairy Sci. 73:2333-2341.

Stephenson, K.A., I.J. Lean, M.L. Hyde, M.A. Curtis, J.K. Garvin, and L.B. Lowe. 1997. Effect of monensin on the metabolism of periparturient dairy cows. J. Dairy Sci. 80:830–837.

Stokes, S.R., and J.P. Goff. 2001. Evaluation of calcium propionate and propylene glycol administered into the esophagus at calving. Prof. Anim. Scientist 17:115–122.

Strang, B.D., S.J. Bertics, R.R. Grummer, and L.E. Armentano. 1998. Effect of long-chain fatty acids on triglyceride accumulation, gluconeogenesis, and ureagenesis in bovine hepatocytes. J. Dairy Sci. 81:728-739.

Studer, V.A., R.R. Grummer, S.J. Bertics, and C.K. Reynolds. 1993. Effect of prepartum propylene glycol administration on periparturient fatty liver in dairy cows. J. Dairy Sci. 76:2931-2939.

Swanson, E. W. 1960. Effect of rapid growth with fattening of dairy heifers on the lactational ability. J. Dairy Sci. 43:377-387.

Swartz, L.A., A.J. Heinrichs, G.A. Varga, and L.D. Muller. 1991. Effects of varying dietary undegradable protein on dry matter intake, growth, and carcass composition of Holstein calves. J. Dairy Sci. 74:3884–3890.

Thonney, M.L., and D.E. Hogue. 1986. Fish meal or cottonseed meal as supplemental protein for growing Holstein steers. J. Dairy Sci. 69:1648-1651.

Tomlinson, D.J., R.E. James, G.L. Bethard, and M.L. McGilliard. 1997. Influence of ration protein undegradability on intake, daily gain, feed efficiency, and body composition of Holstein heifers. J. Dairy Sci. 80:943-948.

Tozer P.R., and A.J. Heinrichs. 2001. What affects the costs of raising replacement dairy heifers: a multiple-component analysis. J. Dairy Sci. 84:1836- 1844.

Tsang, D.S., C.V. Ballard, C.J. Sniffen, R.D. Allshouse, S. Miyoshi, and L. Schlatter. 1997. The effects of NutroCal fed pre- and post-partum on health and performance of multiparous Holstein cattle. J. Dairy Sci. 80 (Suppl.1):209 (Abstr.).

Tucker, H.A. 1981. Physiological control of mammary growth, lactogenesis, and lactation. J. Dairy Sci. 64:1403–1421.

Valentin, S.C., R.C. Dobos, P.A. Lewis, B.D. Bartsch, and R.B. Wickes. 1987. Effect of live weight gain before and during pregnancy on mammary gland development and subsequent milk production of Australian Holstein-Friesian heifers. Aust. J. Exp. Agric. 27:195-204.

Van Amburgh, M., and D.M. Galton. 1994. Accelerated growth of Holstein heifers-effects on lactation. Pp.147 In: Proc. Cornell Nutr. Conf. Feed Manuf., Syracuse, NY. Cornell Univ., Ithaca, NY.

Van Amburgh, M. E., D. G. Fox, D. M. Galton, D. E. Bauman, and L. E. Chase. 1998a. Evaluation of National Research Council and Cornell Net Carbohydrate and Protein systems for predicting requirements of Holstein heifers. J. Dairy Sci. 81:509-526.

Van Amburgh, M.E., D.M. Galton, D.G. Fox, D.E. Bauman, L.E. Chase, H.N. Erb, and R.W. Everett. 1994. Effect of prepubertal growth rate in Holstein heifers on first lactation milk yield. J. Dairy Sci. 77(Suppl. 1):185 (Abstr.).

Van Amburgh, M. E., D. M. Galton, D. E. Bauman, R. W. Everett, D. G. Fox, L. E. Chase, and H. N. Erb. 1998b. Effects of three prepubertal body growth rates on performance of Holstein heifers during first lactation. J. Dairy Sci. 81:527-538.

VandeHaar, M.J. 1997. Dietary protein and mammary development of heifers: Analysis from literature data. J. Dairy Sci. 80(Suppl 1):216 (Abstr.).

VandeHaar, M.J. 1998. Feeding heifers as a long-term investment. Pp. 1. In Proc. Northwest Dairy Nutr. Shortcourse. Jan. 30, Semiahmoo, WA, Washington State Univ.

VandeHaar, M.J., G. Yousif, B.K. Sharma, T.H. Herdt, R.S. Emery, M.S. Allen, and J.S. Liesman. 1999. Effect of energy and protein density of prepartum diets on fat and protein metabolism of dairy cattle in the periparturient period. J. Dairy Sci. 82:1282-1295.

Van Saun, J.R. 1991. Dry cow nutrition: the key to improve fresh cow performance. Vet. Clin. N. Am. 7:599–620.

Van Soest, P.J. 1994. Nutritional ecology of the ruminant. 2nd. ed. Cornell Univ. Press, Ithaca, NY.

Veira, D.M., G.K. Macleod, J.H. Burton, and J.B. Stone. 1980. Nutrition of the weaned Holstein calf. II. Effect of dietary protein level on nitrogen balance, digestibility, and feed intake. J. Anim. Sci. 50:945–951.

Vestergaard, M., K. Sejrsen, J. Foldager, S. Kastrup, and D.E. Bauman. 1993. The effect of bovine growth hormone on growth, carcass composition, and meat quality of dairy heifers. Acta Agric. Scand. 43:165-170.

Waldo, D.R., A.V. Capuco, and C.E. Rexroad, Jr. 1998. Milk production of Holstein heifers fed either alfalfa or corn silage diets at two rates of daily gain. J. Dairy Sci. 81:756-764.

Waldo, D.R., A.V. Capuco, and C.E. Rexroad, Jr. 1989. Replacement heifers growth rates affect milk producing ability. Feedstuffs. 27:15-17.

Waldo, D.R., and L.H. Schultz. 1960. Blood and rumen changes following the intra-ruminal administration of glycogenic material. J. Dairy Sci. 43:496-501 .

Wallace, R.L., G.C. McCoy, T.R. Overton, and J.H. Clark. 1996. Effect of adverse health events on dry matter consumption, milk production, and body weight loss of dairy cows during early lactation. J. Dairy Sci. 79(Suppl. 1):205. (Abstr.).

Waterman, R.C., J.E. Sawyer, F. Valdez, J. Horton, and M.K. Petersen. 2002. Insulin responsiveness improved as glucogenic potential increased in protein supplements fed to young post-partum range beef cows. J. Dairy Sci. 85(Suppl. 1):48 (Abstr.).

Weber, M.S., S. Purup, M. Vestergaard, R.M. Akers, and K. Sejrsen. 2000. Regulation of local synthesis of insulin-like growth factor-I and binding proteins in mammary tissue. J. Dairy Sci. 83:30-37.

Weber, M.S., S. Purup, M. Vestergaard, J. Sondergaard-Andersen, R.M. Akers, and K. Sejrsen. 1999. Contribution of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 (IGFBP-3) to mitogenic activity in bovine mammary extracts and serum. J. Endocrinol. 161:365–373.

Whitlock, B.K., M.J. VandeHaar, L.F. Silva, and H.A. Tucker. 2002. Effect of dietary protein on prepubertal mammary development in rapidly growing dairy heifers. J. Dairy Sci. 85:1516-1525.

Wilkerson, V.A., D.R. Mertens, and D.P. Casper. 1993. Prediction of excretion of manure and nitrogen by Holstein dairy cattle. J. Dairy Sci. 80:3193–3204.

Willett, G.S., J.W., Bernard, R.A. Cady, G.R. Fredricks, D.C. Grusenmeyer, W.H. Madson, R.W. Mathews, and E.L. Thomson. 1992. Dairy heifers enterprise budget. Bull. No. EB1319, Coop. Ext. Serv., Washington State Univ., Pullman, WA.

Wu, Z., and J. T. Huber. 1994. Relationship between dietary fat supplementation and milk protein concentration in lactating cows: a review. Livest. Prod. Sci. 39:141–155.

Young, C.W., V.R. Erdman, and J.K. Reneau. 1985. Animal health and management and their impact on economic efficiency. J. Dairy Sci.68:1593–1602.

Young, J.W. 1977. Gluconeogenesis in cattle: significance and methodology. J. Dairy Sci. 60:1-15.

Zamet, C.N., V.F. Colenbrander, C.J. Callahan, B.F. Chew, R.E. Erb, and N.J. Moeller. 1979. Variables associated with peripartum traits in dairy cows. I. Effect of dietary forages and disorders on voluntary intake of feed, body weight and milk yield. Theriogenology 11:229-244.

BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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